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PYTHIUM ROOT ROT OF MILO[1]

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INTRODUCTION

Milo, one of the grain sorghums (*Sorghum vulgare* Pers.), was considered particularly free from diseases until about 1924 (*28, 34*).[3] Since that time serious losses due to what has come to be known as the "milo disease" have occurred in the sorghum-growing areas of the southern Great Plains. Preliminary investigations of a disease attacking milo were carried on at substation no. 12 of the Texas Agricultural Experiment Station, Chillicothe, Tex.[4] Milo plants were stunted and produced little or no grain. Rotting of the central shoot occurred in some plants. Bacteria and *Fusarium moniliforme* Sheldon were repeatedly isolated from these plants but did not produce the disease symptoms when used for inoculation. Because of the presence of chinch bugs (*Blissus leucopterus* Say) at Chillicothe and the similarity of chinch bug injury on milo to the milo disease, most of the field work was transferred in 1930 to the Garden City substation of the Kansas Agricultural Experiment Station, Garden City, Kans., where a distinct and constantly recurring disease was attacking milo and where chinch bugs were not present to confuse the disease symptoms. Since that time field and greenhouse studies of this disease have been conducted at the Arlington Experiment Farm, Arlington, Va. (near Washington, D. C.), at Manhattan and Garden City, Kans., and at Dalhart and Chillicothe, Tex.

A preliminary report was published in 1932 by Elliott, Wagner, and Melchers (*12*). In 1934, Melchers and Parker (*21*) called attention to the importance of the disease in Kansas and the new resistant selections made by Wagner. In 1934, Myers (*22*) reported the results on the effects of fertilizers on the disease. Wagner (*35*) has recently published a paper on varietal differences in susceptibility and on his new resistant selections. The results of the investigations reported in the present paper show that the milo disease is similar in cause and symptoms to the root rots of other plants of the grass family.

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[2] The writers gratefully acknowledge the assistance given by Dr. John H. Parker, professor of agronomy, Department of Agronomy, Kansas State College of Agriculture and Applied Science, in the work conducted in Kansas.

[3] Reference is made by number (italic) to Literature Cited, p. 833.

[4] Work at the Chillicothe station was carried on in cooperation with Superintendent J. P. Quinby.

During the past 10 years *Pythium* and related genera have been recorded as the causes of root rots of important crop plants of the grass family in widely separated localities and countries. Root rots of wheat and other cereals in Canada (*30*, *31*, *32*, *33*), of corn in the Central States (*1*, *17*, *29*) and in the Philippine Islands (*27*), and of sugarcane in the Southern States (*24*), Hawaii (*4*, *5*, *6*, *7*), and other cane-growing countries have been attributed to these fungi. Recent comparative studies (*26*) of 70 isolates from roots of these hosts have led to the conclusion that (*26*, *p. 191*) "all these cosmopolitan and obviously variant types had enough distinctive characters in common to be classified most usefully under a broadened concept of the species *Pythium arrhenomanes*." The literature on these root rots has been recently reviewed by Carpenter (*4*) and by Rands and Dopp (*26*). In all cases the fungus is soil-borne and attacks the uninjured cortex of young roots, destroying much or all of the root system. The plants are stunted and the yield is reduced.

In 1929, Edgerton, Tims, and Mills (*11*) reported that sorghum plants in the greenhouse developed root rot when inoculated with the *Pythium* isolated from sugarcane roots. The variety of sorghum was not given.

The results of studies of pythium root rot of sorghum in the field, known as the "milo disease", are presented here.

MATERIAL AND METHODS

Studies of the milo disease have been conducted in the field and in the greenhouse. The results of experiments conducted in the greenhouse on the nature and control of the disease have agreed with those obtained in field trials. Symptoms are definite and clean-cut for susceptible varieties. Varieties that are resistant in the field are resistant in the greenhouse also.

The soil used in the greenhouses at Manhattan, Kans., and at Arlington Farm was obtained from the Garden City substation of the Kansas Agricultural Experiment Station or from other points mentioned in Kansas, Texas, and Oklahoma. Infested soil was taken from plots at Garden City that had previously grown diseased plants. Noninfested soil was obtained from other areas on the Garden City substation farm, where sorghums had not been grown for a number of years and where the disease had never been observed.

In the greenhouse studies at Manhattan, Kans., soil-temperature tanks and 1-gallon earthen jars were used, although wooden flats were found satisfactory for certain phases of the investigations. The jars were watered through a glass tube inserted in the soil in the center of the jar, and the soil moisture was maintained at the desired percentage by weighing the jars at intervals and adding water as required. As a rule, the soil moisture was maintained at a point between 17 and 19 percent of the dry weight of the soil. Twenty-five to thirty-five seedlings were generally grown in a jar. At Arlington Farm plants were grown in earthen flowerpots 6 to 10 inches in diameter, with 10 seedlings to a pot, or in soil-temperature tanks. In making inoculations the soil was thoroughly wet when the inoculum was added, and seed was planted at once. The temperature of the greenhouses was maintained between 21° and 35° C.

The varieties and selections used in most of the tests involving disease transmission were Dwarf Yellow milo (C. I.[5] 332), Club (C. I. 901), darso (C. I. 615), Fargo milo (C. I. 809), and various resistant selections from Dwarf Yellow milo (C. I. 332) made by one of the writers (Wagner).

The seed at Manhattan was treated with copper carbonate or Semesan in order to obtain better stands. This treatment did not prevent infection by the causal organism. In some experiments the seed was first sprouted and then planted to assure a good stand. At Arlington Farm, unless otherwise stated, seed was treated with Germisan (0.3 g in 100 cc of water) for 15 minutes and then thoroughly washed.

At Manhattan, various combinations of sorghum varieties were planted in jars, except in a few instances noted. The susceptible Dwarf Yellow milo and resistant Club were always grown in the same jar, so that all soil conditions would be the same for both varieties. A selection of resistant Dwarf Yellow milo was also included in some of the jars. The surface of the soil in each jar was divided into halves, thirds, or quarters by means of small pot labels, thus giving a boundary between varieties. At Arlington Farm each variety was grown in a separate pot.

THE DISEASE

HISTORY AND SYMPTOMS

Pythium root rot of milo was first observed in Kansas in 1926 at the Garden City substation, where milo had been grown continuously for several years in six $\frac{1}{20}$-acre plots. The disease was confined at first to small areas which increased in succeeding years. In 1928, the six plots were planted to resistant kafir. The disease of previous years did not develop and a good crop was harvested. In 1929, the same six plots were again planted to Dwarf Yellow milo. By the end of the season all of the plants were diseased or dead. None grew more than 12 to 15 inches tall and no heads were produced. As the land was no longer suitable for the fertilizer experiment that had been laid out, it was decided to use it as a plot for studying the cause of the milo disease and methods of control. It was planned to grow milo indefinitely in one of the six $\frac{1}{20}$-acre plots and to use the other plots for testing varieties of sorghum for relative resistance to the disease and for observing the effects of fallow and of crop rotations upon the length of time the disease-producing agent remained in the soil. A portion of the varietal series on this infested soil is shown in figure 1.

Plants in the field at Garden City usually make a growth of 8 to 12 inches before disease symptoms appear. There is first a slight rolling or drooping of the leaves, accompanied by retarded growth. The older leaves begin to turn yellow at the tips and along the margins. This yellowing and drying progresses until the entire plant is affected and dies without heading (fig. 2). The discoloration of the dying plants resembles a mild "firing" injury caused by drought or alkali. Plants less heavily infected may produce small heads. There is no abnormal tillering and no soft rot of the stalk or crown except as secondary infection may occur.

[5] C. I. refers to accession number of Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

Sometime before these symptoms appear on the above-ground parts of milo plants, symptoms of the disease appear on the roots. When plants are not more than 3 inches tall the roots may show a characteristic water-soaked brown or red discoloration of the cortex and vascular tissue. As the disease progresses a soft rot destroys much or all of the fine root system, while the larger roots turn brown or dark red, especially in the central cylinder. Dark-red discoloration of the tissue at the base of the crown (fig. 3) is also characteristic,

FIGURE 1.—Plantings of resistant and susceptible varieties of sorghum on naturally infested soil at Garden City, Kans., 1930. *A*, *a* and *b*, Dwarf Yellow milo; *c*, Fargo milo; *d*, Early White milo; *e*, Kalo. *B*, *a*, Dwarf Yellow milo × Pink kafir (C. I. 903); *b*, Double Dwarf milo; *c*, Dwarf Yellow milo.

and this discoloration extends into the roots and to some extent up through the vascular bundles of the stalk.

At Garden City, field symptoms of the disease are evident 30 to 35 days after planting. Milo planted May 25, 1931, showed the first symptoms of disease on June 30; the June 5 planting, on July 5; and the June 16 planting, on July 21. All of the plants died early and the disease was as severe in one planting as in another.

GEOGRAPHIC DISTRIBUTION

Milo showing similar disease symptoms as at Garden City has been observed at Hays, Kans., Tucumcari, N. Mex., and at widely separated points in Texas, Oklahoma, and possibly California. In 1930,

the disease occurred in small areas on the United States Dry Land Field Station at Dalhart, Tex., and in commercial fields of Dwarf Yellow milo near Robstown, Tex. By 1933 the disease had become

FIGURE 2.—Diseased and healthy plants of Custer and Dwarf Yellow milo grown at Garden City, Kans., 1930: *A*, Diseased plant of Custer; *B*, healthy plant of Custer; *C*, diseased plant of Dwarf Yellow milo; *D*, healthy plant of Dwarf Yellow milo.

generally distributed over the station at Dalhart; and near Robstown, fields that had not shown the disease in 1930 were reported so badly infested that it was not possible to produce a crop of milo.

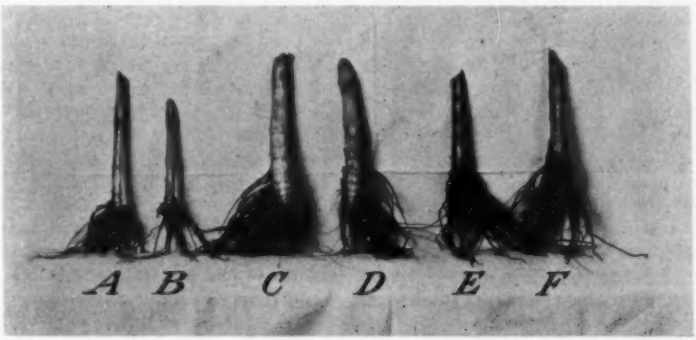

FIGURE 3.—Longitudinal sections of diseased (*A, B, E, F*) and healthy (*C, D*) plants of Dwarf Yellow milo, Garden City, Kans., 1930.

In 1932, diseased milo plants showing the same symptoms as at Garden City were found widely distributed over the station grounds at Tucumcari. In 1935 the disease was present in large areas in the Ballinger area, Runnels County, Tex. It has also been present for several years in certain plots at Woodward, Okla.

In 1935, diseased Double Dwarf milo plants from commercial fields in the lower Sacramento Valley of California showed the characteristic dwarfing, lack of heading, and reddening of roots and crown. The disease in California, however, has been attributed to *Fusarium* injury (*16*).

No survey has been made to determine the extent and distribution of the disease in Kansas, Texas, or other States. In fields where the disease has once occurred it is a limiting factor in milo production.

The disease has never been found, as far as known, on newly plowed sod. It is not associated with poorly drained soils, alkali spots, or lack of fertility in the soil. In most cases it has appeared in soils continuously cropped to milo.

METHODS OF TRANSMISSION

Field observations and studies of the milo disease have given no indication of transmission of the disease through the seed. Early experiments at Manhattan and at Arlington Farm have shown that the disease can be transmitted by means of infected plant refuse, infested soil, and water leachings.

When diseased crowns and roots of milo plants were washed free of all soil, ground into small pieces, and added to noninfested soil, 50- to 100-percent infection was obtained. When noninfested soil planted to susceptible Dwarf Yellow milo was watered with leachings from infested soil filtered through filter paper or with clear decanted liquid from infested soil, no disease appeared in the first crop of plants; in the second and third crops in the same jars, receiving only water leachings, 100-percent infection resulted.

Small amounts of infested soil were more effective in transmitting the disease than either the water leachings or the infected roots. The smallest amount of infested soil used—5 g in a 6-inch pot (about 2,000 kg)—produced infection in 89 percent of the plants, while 10, 15, 20, and 25 g at the end of 6 weeks produced 100-percent infection.

In connection with the experiments at Manhattan mentioned above, in which leached, decanted liquid was used to water plants, a similar experiment was conducted in which the liquid was passed through a Berkefeld filter. This was used for watering plants growing in noninfested soils. One pound of infested Garden City soil was mixed with about 1,000 cc of water, and the material was filtered through unglazed-clay pressure filters. This liquid was then filtered through a fine porcelain Berkefeld filter, according to the method described by McKinney (*20*). Infested soil was used only once for water leaching, after which new lots were used. Before the Berkefeld-filtered water was used, samples were streaked on potato-dextrose agar in order to test for the presence of organisms. In most cases organisms failed to appear on the agar, but in several of the cultures incubated for about 1 week colonies of a reddish, rod-shaped bacterium appeared. This bacterium, however, did not seem to be at all pathogenic to the Dwarf Yellow milo seedlings.

The filtrate was then used to water susceptible Dwarf Yellow milo plants growing in disease-free soil and sterilized soil from Garden City and in Manhattan garden soil free of the disease-producing factor. Although three successive crops of milo were grown in the various soils which were watered with filtrate from the Berkefeld filters, in all

cases the disease failed to develop, indicating that the milo disease is not caused by a filterable virus carried in the soil.

BEHAVIOR OF MILOS IN INFESTED SOIL IN GREENHOUSE

Plants of susceptible varieties grown in infested soil in the greenhouse succumb to the milo disease at an earlier stage than those grown in the field. When the plants are 4 to 6 inches high, the lower leaves turn yellow and begin to dry at the tips, and the drying progresses until the entire plant shrivels and dies. Sometimes one or two leaves remain green and the plant struggles along for several weeks. The

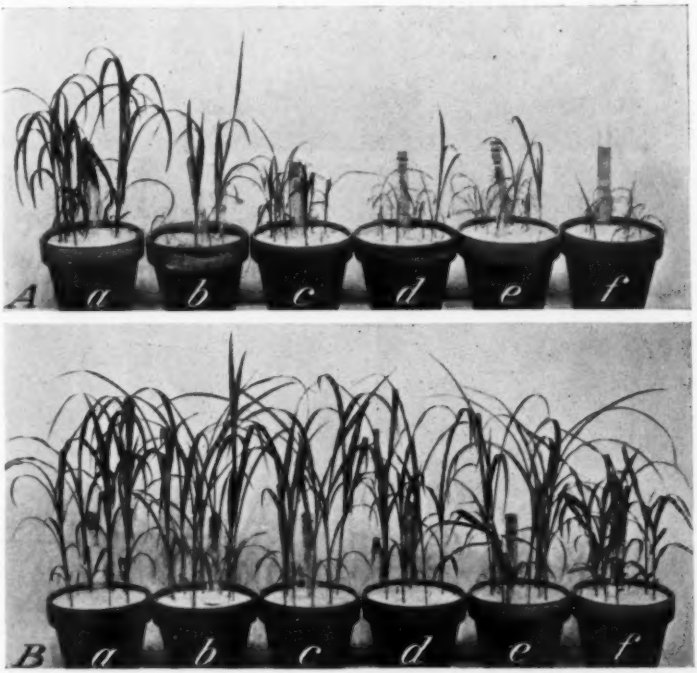

FIGURE 4.—*A*, Resistant and susceptible sorghum varieties grown in naturally infested Garden City soil in the greenhouse at Arlington Experiment Farm, 1931–32; *a*, Fargo milo; *b*, Double Dwarf milo; *c*, Beaver milo; *d*, Dwarf Yellow milo; *e*, Dwarf White milo; *f*, darso. *B*, Resistant and susceptible sorghum varieties grown in noninfested Garden City soil in the greenhouse at Arlington Farm, 1931–32: *a*, Fargo milo; *b*, Double Dwarf milo; *c*, Dwarf White milo; *d*, Beaver milo; *e*, Dwarf Yellow milo; *f*, darso. Planted November 27, 1931; photographed January 19, 1932.

root symptoms of diseased plants appear the same as in the field. The fine roots are destroyed while the larger ones and the crown turn dark red. Infected small secondary roots become flaccid and are full of mycelium, lobulate sporangia, and oospores. These flaccid roots soon decay and are readily broken off when diseased plants are removed from the soil. If some of the roots of susceptible plants in infested soil are able to grow through the bottoms of pots or boxes into noninfested soil of the greenhouse beds, the plants frequently recover from the disease.

Milos growing in infested Garden City soil in the greenhouse are shown in figure 4, *A*. The same milo varieties in noninfested Garden City soil are shown in figure 4, *B*. Fargo "milo" is resistant and shows no disease symptoms. The other milos show varying degrees of susceptibility in infested soil, and darso is very susceptible.

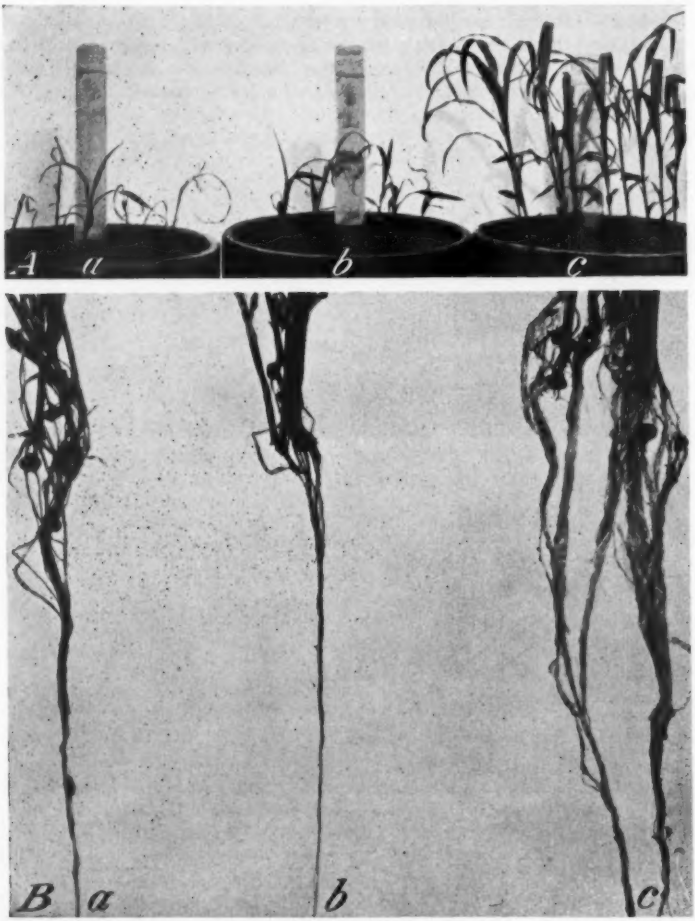

FIGURE 5.—Darso (C. I. 615) grown in naturally infested Garden City soil (*A*, *a*, *b*) and in sterilized Arlington Farm soil (*A*, *c*) at Arlington Experiment Farm. *B*, *a* and *b*, Roots of plants grown in naturally infested Garden City soil; *c*, roots of plants grown in sterilized Arlington Farm soil. Planted October 31; photographed November 26, 1930.

The results of growing a susceptible variety of sorghum in infested Garden City soil and in sterilized Arlington Farm soil are shown in figure 5. These photographs were taken less than 4 weeks after planting and show the condition of the roots at the time the young plants were beginning to show yellowing and drying of leaf tips. The

root systems in infested soil are much reduced, and the roots that remain are discolored and partly dead. The dark-red discoloration of the base of the stalk is shown in figure 5, *B*, *a* and *b*.

A comparison of the results of growing susceptible Dwarf Yellow milo in noninfested and infested soil from five different localities is given in table 1. Plants in Chillicothe soils grew much larger than those in any other soil. The roots were not diseased, and *Pythium* was not reisolated from these plants. The earlier disease symptoms in milo grown in Chillicothe soil were apparently not due to the same cause as the disease symptoms on plants grown in Garden City soil. This Chillicothe soil was apparently not infested with *Pythium*.

TABLE 1.—*Results of growing susceptible milo for 6 weeks, in sterilized, noninfested, and infested soils from five different localities, at Arlington Experiment Farm, 1931–32*

Variety	Soil		Pots	Seeds planted	Seeds germinated	Plants diseased or dead [1]	Average height of plants
	Original locality	Treatment					
			Number	*Number*	*Percent*	*Percent*	*Inches*
Dwarf White milo.	Arlington, Va. [2]	Noninfested	6	60	98	0	12
		Sterilized	6	60	98	0	18
	Garden City, Kans.	Noninfested	6	60	97	0	10
		Infested	6	60	98	100	7
		Sterilized	6	60	98	0	18
	Chillicothe, Tex.	Noninfested	3	30	96	0	23
		Infested (?) [3]	3	30	94	0	20
		Infested subsoil (?) [4]	3	30	100	0	33
	Robstown, Tex.	Noninfested	3	30	100	0	24
		Infested	3	30	100	100	6
Dwarf Yellow milo.	Dalhart, Tex.	Noninfested [5]	1	10	100	[5] 100	9
		Infested	1	10	90	100	4

[1] Because of *Pythium* infection.
[2] Greenhouse soil at Arlington Experiment Farm.
[3] Soil from Chillicothe station in which diseased milo plants had grown.
[4] Subsoil from Meharg farm, adjacent to the Chillicothe station, in which diseased milo plants had grown.
[5] Soil as nearly free from infestation as possible was selected. The infestation was very generally distributed at Dalhart.

The percentage of diseased or dead plants in the infested soil from Garden City, Robstown, and Dalhart, as well as the comparatively small size of the plants, shows the effects of the disease in the infested soils. In soil from Dalhart labeled noninfested, all the plants showed *Pythium* injury, but the size of the plants in this soil shows that the injury was not so severe as in the infested soil. The infestation was so generally distributed at Dalhart that it was not possible to obtain soil free from *Pythium*.

THE CAUSAL ORGANISM

ISOLATIONS

When pieces of diseased milo roots are plated on beef-peptone, potato-dextrose, corn-meal, or sorghum-extract agar, *Fusarium moniliforme* appears on the plates and outgrows the other organisms. However, none of the *Fusarium* cultures when used for inoculation in the field or greenhouse produced the typical disease symptoms on milo at Manhattan or Garden City or at Arlington Farm. This organism has been reported as associated with seedling blight of sorghum (*15*), which is different from milo root rot.

When diseased root tips or small roots were plated on water agar (*9*) *Pythium* was readily isolated. The roots were washed in sterile water and allowed to stand in water several hours or overnight in the refrigerator. Excess moisture was pressed out and the roots were plated on 2-percent water agar. *Pythium* hyphae, visible by transmitted light, grew more rapidly than other fungi, and transfers of tips of hyphae were made to suitable media. By this method *Pythium* was isolated from diseased milo plants growing in naturally infested Garden City soil in the greenhouse at Arlington Farm in March 1932.[6] Subsequently *Pythium* cultures were isolated from mesocotyls and reddened tissue from the interior of the crowns of diseased plants as well as from water-soaked rootlets. The same *Pythium* has repeatedly been isolated from diseased roots of plants grown in infested soil in the field and in the greenhouse. This fungus has been isolated also from plants grown in the greenhouse at Arlington Farm in infested soil from Robstown and Dalhart, as well as from Garden City. In the fall of 1932, *Pythium* was isolated from diseased milo roots at Manhattan. *Pythium arrhenomanes* has also been found in root materials taken from milo plants grown in infested soils from Ballinger, Tex., and Woodward, Okla. Isolation methods described by Machacek (*19*) and Brown (*3*), as well as those described by Drechsler (*9*), were used at Manhattan. Altogether, more than 200 isolations have been made.

TAXONOMY

The *Pythium* cultures isolated from diseased milo plants have been identified by Rands and Dopp and also by Drechsler as *P. arrhenomanes*. They show the crook-necked antheridia and the remote connection between male and female organs characteristic of this species. Grown on water agar, the fungus is visible by transmitted light. Lobulate sporangia are formed abundantly on corn-meal (fig. 6, *A*) and on water agar (fig. 6, *B*) and in the cortex of flaccid rootlets (fig. 7, *A* and *B*). On corn-meal agar, potato-dextrose agar, and corn-meal agar plus grated carrot, there is considerable aerial growth of a dense white mycelium around the outer margins of the plates. Hyphae are 1μ to 5μ in diameter. Oogonia and antheridia develop abundantly on corn meal-carrot-humic acid agar (fig. 8) and also occur in infected rootlets. The following are the diameters of oogonia of six isolates from field infections, grown on corn meal-carrot-humic acid agar (*25*) at room temperature: No. 1, 28.3μ; no. 2, 28.5μ; no. 3, 28.8μ; no. 4, 27.2μ; no. 5, 28.5μ; and no. 6, 31.8μ; average, 28.9μ. One hundred oogonia were measured in each case from plates 2 to 7 days old. In size of oogonia these strains would fall in the middle frequency classes figured by Rands and Dopp (*26*). Oospores in the roots measured 27.4μ, while on corn-meal agar they were slightly larger, measuring 29.7μ. One hundred oospores were measured in each case. The walls ranged from 1.0μ to 2.0μ in thickness. The number of crook-necked antheridia attached to each oogonium ranged from 3 to 10, the exact number being difficult to determine. Rands and Dopp (*26*) state that a considerable proportion of the 70 isolates studied by them had 4 to 10 visible antheridia instead of the 15 to 20

[6] The senior author is indebted for advice and suggestions to R. D. Rands and Ernest Dopp, of the Division of Sugar Plant Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.

mentioned in Drechsler's original description and that the range should be changed to 4 to 20 to include all strains.

This species was first reported by Johann, Holbert, and Dickson (*17*) in 1928 as causing seedling blight and root rot of dent corn in

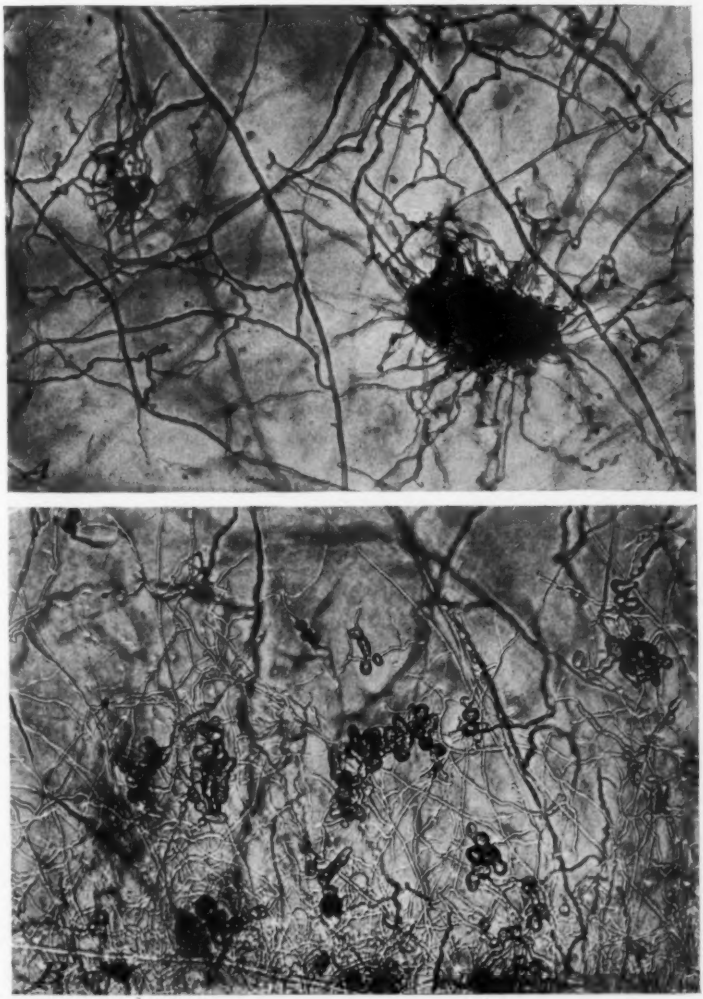

FIGURE 6.—Cultures of *Pythium arrhenomanes*. *A*, Lobulate sporangia growing on corn-meal agar, pH 4.5, isolation no., Dwarf Yellow milo 8; *B*, lobulate sporangia growing on water agar, isolation no., Dwarf Yellow milo 11. Plated April 15; photographed April 17, 1935. × 150.

Wisconsin and Illinois and was described and named *Pythium arrhenomanes* by Drechsler (*8*). In 1929, Rands (*24*) reported it as causing root rot of sugarcane in Louisiana and Florida. In 1934, Rands and Dopp (*26*) discussed the taxonomy of the species and described in

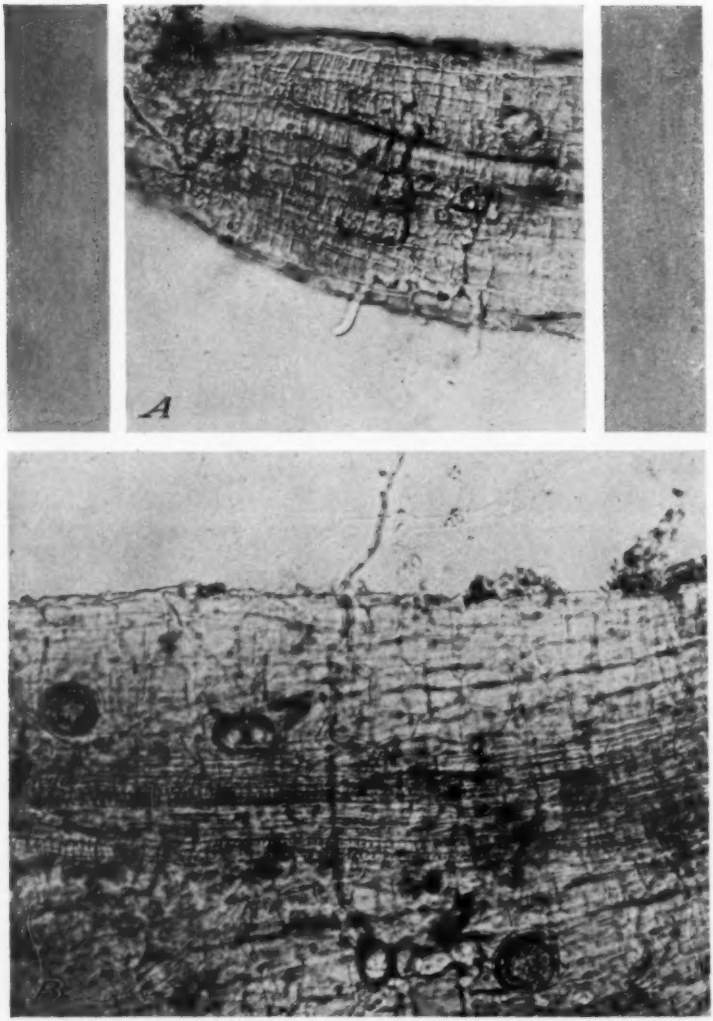

FIGURE 7.—*A* and *B*, Oogonia and lobulate sporangia of *Pythium arrhenomanes* in the young roots of Dwarf Yellow milo plants grown in naturally infested Garden City soil at Arlington Experiment Farm, 1934. × 534.

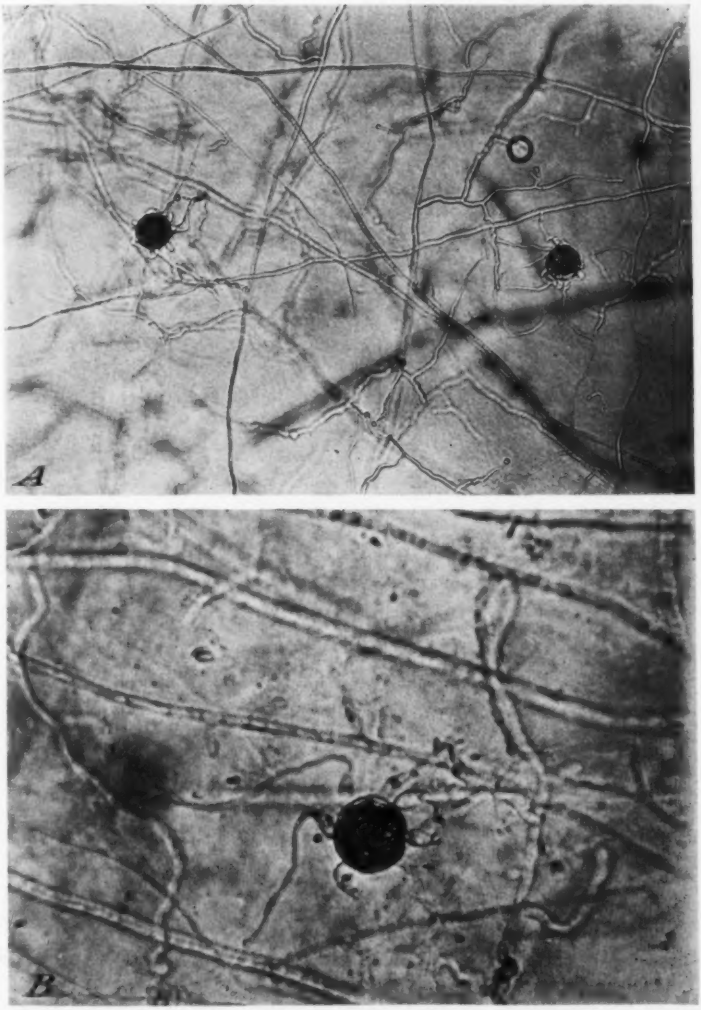

FIGURE 8.—Cultures of *Pythium arrhenomanes*. Oogonia and antheridia growing (*A*) on water agar (isolation no., Dwarf Yellow milo 7), × 240, and (*B*) on corn-meal agar (isolation no., Dwarf Yellow milo 7). × 372. Plated April 15; photographed April 17, 1935.

detail the morphological and cultural characters. The 70 strains studied from corn, sugarcane, and wheat from widely separated localities were all placed under one species, *P. arrhenomanes*.

To determine the optimum temperature for the growth of the fungus in culture, small squares (about 5×5 mm) of agar cultures of five isolations were transferred to corn-meal agar plates. Two plates of each strain were held for 48 hours in constant-temperature chambers at

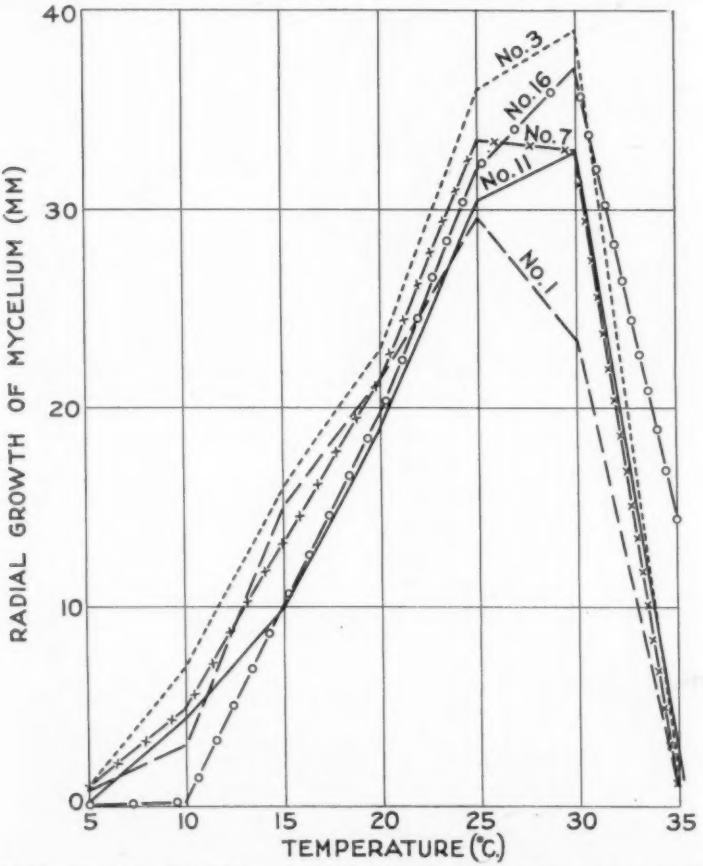

FIGURE 9.—Mycelial growth of *Pythium arrhenomanes* when held for 48 hours at temperatures ranging from 5° to 35° C. Five strains isolated from milo and grown on corn-meal agar, pH 5.6.

each of the following temperatures: 5°, 10°, 15°, 20°, 25°, 30°, 35°, and 40° C. The results from the 40° chamber were discarded because of defective control. Figure 9 gives the results of this test. The fungus grew most rapidly at 25° to 30°. Above 30° the growth dropped rapidly. Below 25° growth decreased with the decrease in temperatures from 20° to 15° and 10°.

The organism enters the unwounded cortex of young roots, causing water soaking, browning, and reddening of the tissue. The cortex is

destroyed and the fungus penetrates the vascular tissue, where it causes a red discoloration.

PATHOGENICITY

Greenhouse Tests

The first isolations of *Pythium arrhenomanes* from milo were used for inoculation the following month in the greenhouse at Arlington Farm and were found to be pathogenic to milo. The pathogenicity

FIGURE 10.—Susceptible Dwarf Yellow milo grown at Arlington Experiment Farm in Garden City soil that had been steamed 4 hours at 20 pounds pressure on two successive days and then had been inoculated at planting time with young agar cultures of *Pythium arrhenomanes:* Group *A*, Checks, no inoculation; groups *B*, *C*, and *D*, inoculated. Planted and inoculated January 26; photographed March 12, 1934.

of these and later isolates was tested by growing the cultures on corn-meal agar, corn meal-carrot-humic acid agar, or potato-dextrose agar and mixing the broken-up cultures with the thoroughly moistened soil at the time the seed was planted.

Sixteen series of inoculations in the greenhouse at Arlington Farm during the period 1932–34, with *Pythium arrhenomanes* isolated from

FIGURE 11.—Susceptible Dwarf Yellow milo grown at Arlington Experiment Farm in Garden City soil that had been steamed 2 hours at 20 pounds pressure and then had been inoculated at planting time with cultures of *Pythium arrhenomanes: A*, Check, sterile medium added to soil; *B*, *C*, *D*, and *E*, *P. arrhenomanes* added to soil. Planted and inoculated April 3; photographed April 26, 1933.

diseased milo plants, consistently produced stunting of the plants, rotting of the roots, characteristic reddening of roots and crown, and in many cases premature death of the plants. Germination was reduced to a greater extent than in naturally infested soil, and some seedling blight occurred. The two series of inoculations made in 1933 and 1934 are representative of the entire series. The results are given in table 2 and figures 10 and 11.

TABLE 2.—*Results of growing susceptible Dwarf Yellow milo, in steamed Garden City soil inoculated with Pythium arrhenomanes, at Arlington Experiment Farm, 1933–34*

EXPERIMENT IN 1933-34 [1]

Original host	Culture no.	Seeds		Plants		Green weight of tops	
		Planted [2]	Germinated	Diseased	Dead	Total	Average reduction
		Number	*Percent*	*Percent*	*Percent*	*Grams*	*Percent*
Milo [3]	1	10	80	100	0	1.6	
	2	10	60	100	0	3.1	
	3	10	30	100	0	2.8	
	4	10	80	100	0	4.1	
	5	10	80	100	0	3.2	
	6	10	70	57	14	3.1	
Average		10	66.7	92.8	2.3	3.0	90.6
Milo [4]	7	10	80	100	0	2.0	
	8	10	90	100	0	3.5	
	9	10	20	0	0	10.9	
	10	10	50	60	20	2.0	
	11	10	80	100	0	3.3	
	12	10	60	100	17	4.1	
	13	10	100	100	0	2.3	
	14	10	90	100	11	2.4	
	15	10	60	84	0	1.5	
	16	10	80	75	25	1.2	
	17	10	90	100	0	2.3	
	18	10	20	100	0	4.5	
	19	10	70	100	0	2.1	
	20	10	70	100	0	4.4	
	21	10	10	100	0	2.0	
	22	10	60	50	34	1.5	
	23	10	60	83	17	1.2	
	24	10	70	86	14	4.0	
Average		10	64.4	85.4	7.7	3.1	90.3
Sugarcane [5]	25	10	80	100	0	5.1	
	26	10	80	88	0	6.8	
Average		10	80	94	0	6.0	81.2
Checks [6]		10	92	0	0	31.9	

EXPERIMENT IN 1934 [7]

Milo [3]	1	30	80	82	4	1.97	
	2	30	53	82	18	1.09	
	3	30	53	87	13	1.32	
	4	30	17	50	17	.17	
	5	30	73	73	27	1.71	
	6	30	67	86	14	2.00	
Average		30	57.2	76.7	15.5	1.38	86.4
Milo [4]	7	30	50	46	54	.65	
	8	30	67	85	15	2.12	
	9	30	77	0	0	7.15	
	10	30	53	70	30	1.11	
	11	30	57	87	18	.75	
	12	30	47	60	40	.59	
	13	30	63	90	10	.68	
	14	30	20	22	11	.20	
	15	30	27	42	24	.29	
	16	30	67	65	36	.67	
	17	30	63	81	20	.94	
	18	30	30	92	8	.51	
	19	30	40	92	8	.53	
	20	30	20	72	28	.19	
	21	30	50	83	17	1.13	
	22	30	63	48	52	.68	
	23	30	30	83	17	.24	
	24	30	37	64	36	.32	
Average		30	47.8	65.7	23.6	1.04	59.8
Checks [8]			85	1.1	.9	10.20	

[1] Soil was inoculated and seed sown Nov. 25, 1933; disease data taken Dec. 27, 1933; and green weight of tops taken Jan. 5, 1934.

[2] In the 1933–34 experiment, 10 seeds were sown in each test; in the 1934 experiment, 30 seeds were sown in each test, 10 in each of 3 pots.

[3] Diseased plants were grown in infested field plots in Garden City.

[4] Diseased plants were grown in the greenhouse at Arlington in naturally infested soil from Garden City.

[5] Cultures were kindly furnished by Ernest Dopp.

[6] Average from plants grown in noninoculated soil in 10 pots in each of which 10 seeds were sown.

[7] Soil was inoculated and seeds sown Jan. 26, 1934; disease data taken Mar. 10; green weight of tops taken Mar. 17.

[8] Average from plants grown in noninoculated soil in 12 pots in each of which 10 seeds were sown.

The symptoms of disease in inoculated plants were the same as in plants grown in the greenhouse in infested soil from the Garden City station. The differences in green weight of tops of inoculated diseased plants and uninoculated controls show the degrees of stunting in inoculated plants. When the plants were 3 to 4 inches tall the tip of the first leaf turned yellow. These leaves gradually dried and the yellowing and drying progressed to the inner leaves until the plants were killed. The cortex and vascular tissue of small roots decayed until there were few or no normal root tips left on these plants.

The disease was never so severe from inoculation as in the naturally infested soil, but the stunting, root rot, and other disease symptoms were typical. Infested plants all remained stunted unless the roots were able to grow into clean soil, when they recovered and grew into healthy plants. Check plants grew normally in steam-sterilized Garden City soil. Inoculations made by spraying soil and seed with water suspensions of *Pythium* caused stunting of plants. *Pythium* cultures were reisolated from inoculated diseased plants but not from controls grown in sterilized soil.

The *Pythium* used for inoculum in the Manhattan studies showed injury to Dwarf Yellow milo (1) by preventing germination of the seed and (2) by killing the sorghum seedlings as they emerged or after the plants had produced two or three leaves but were not over 6 inches tall.

Inoculations at Arlington Farm with cultures of *Pythium arrhenomanes* from sugarcane [7] produced the typical disease symptoms on milo, but these cultures were not so virulent as those more recently isolated from milo.

During the winter of 1934 milo plants growing in sterilized Garden City soil in the greenhouse at Arlington Farm were inoculated when 8 inches high with agar cultures of *Pythium arrhenomanes* placed on the roots. These plants soon stopped growing, the leaves turned yellow, and the plants died. The roots of the inoculated plants were red, and root tips and cortices were destroyed. Control plants continued to grow normally.

Similar inoculations were made on 6-inch Dwarf Yellow milo plants with the same results. Corn-meal cultures of *Pythium arrhenomanes* were stirred into the soil in the center of the pot to a depth of about 6 inches. Two weeks later plants in inoculated pots showed yellowing of leaf tips and lower leaves. When the experiment was ended 11 weeks later the inoculated plants were only half the size of the uninoculated. The average green weight of 24 plants grown in noninoculated soil was 222.6 g and that of 34 plants grown in inoculated soil was 57.3 g, the percentage reduction being 74.2.

In another experiment Dwarf Yellow milo seed was planted in three boxes of sterilized Garden City soil. One side of each box had a removable glass slide. When the plants were 3 inches tall inoculations were made with pieces of agar cultures of *Pythium arrhenomanes* placed on uninjured roots growing against these glass slides in two of the boxes, and with pieces of sterile agar placed on roots of plants in the third box. A week later the inoculated roots had turned red and were water-soaked and dead where they were in

[7] The cultures were supplied by Ernest Dopp, of the Division of Sugar Plant Investigations, Bureau of Plant Industry.

contact with the cultures. Pure cultures of *Pythium* were reisolated from these roots.

The infection progressed until all three plants in box no. 1 were dead or dying and the roots were red and decayed. In box no. 2 some of the roots had been killed but other roots had developed and the plants had outgrown the disease and were apparently healthy. In the check (box no. 3) the plants were all healthy.

Johann et al. (*17*) found that temperature and moisture were important factors in the development of pythium root rot. After inoculation of yellow dent corn with *Pythium arrhenomanes* at different temperatures, the reduction in germination, in number of plants at the end of the experiment, and in dry weight of tops was greatest at low temperatures, 12° and 16° C., as compared with higher temperatures such as 20°, 24°, 28°, and 32°. Flor (*13*) found the same to be true for pythium root rot of sugarcane.

Similar experiments were conducted in constant-temperature tanks at Arlington Farm to test the effects of soil temperatures on the development of pythium root rot of milo. The temperatures used were 15°, 20°, 25°, and 30° C. Garden City soil of pH 7.6 was steamed for 4 hours at 20 pounds pressure. Dwarf Yellow milo seed was treated for 10 minutes in a 1 to 1,000 solution of bichloride of mercury and thoroughly washed. Corn meal-carrot-humic acid agar cultures were mixed with the soil at planting time. Two containers of uninoculated controls were included at each temperature. The results of this inoculation are given in table 3. They agree with the results of inoculations on corn and sugarcane at constant temperatures. The greatest reduction in percentage germination, number of healthy plants, and growth of plants occurred at the lowest temperature. During the first 2 weeks after emerging, the milo plants grew rapidly at 30°, the inoculated plants being almost as tall as the controls. At 25° the inoculated plants were 2 to 3 inches shorter than the controls, and at 15° and 20° they were only about half the size of the controls. Cultures of *Pythium arrhenomanes* were reisolated from the roots of these diseased plants.

TABLE 3.—*Results of growing Dwarf Yellow milo, in steamed soil inoculated with Pythium arrhenomanes at planting time, in soil-temperature tanks at 4 temperatures, at Arlington Experiment Farm, Arlington, Va., 1934*

Soil from—	Temperature	Seeds germinated [1]		Reduction following inoculation	Plants healthy at end of experiment		Reduction following inoculation	Green weight of tops		Reduction following inoculation
		Control	Inoculated		Control	Inoculated		Control	Inoculated	
	°C.	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Grams*	*Grams*	*Percent*
Garden City	15	75	28.3	46.7	93.3	41.2	52.1	16.9	0.4	97.7
	20	85	60.2	24.8	94.1	54.0	40.1	126.2	6.1	95.2
	25	90	63.3	26.7	94.4	78.9	15.5	180.5	45.1	75.0
	30	85	66.7	18.3	94.1	70.0	24.1	225.5	165.6	26.6
Arlington Farm	15	60	20.0	40.0	100.0	75.0	25.0	1.5	.1	92.4
	20	80	51.7	28.3	87.5	48.4	39.1	16.9	.8	95.3
	25	80	66.7	13.3	87.5	25.0	62.5	128.6	15.7	87.8
	30	85	80.0	5.0	94.1	47.9	46.2	190.5	96.2	49.5

[1] In each test, 20 seeds were planted in noninoculated soil and 60 in inoculated soil.

No inoculations were made in soil in which a constant moisture content was maintained, but repeated inoculations in the greenhouse showed that when the soil was thoroughly wet at the time the inoculum was added the percentage of infected plants was much higher than when inoculations were made in soil of a lower moisture content.

A similar experiment was carried on at Manhattan to determine the reaction of susceptible milo plants to *Pythium* at different temperatures. Six tanks, ranging in temperature from 16° to 36° C., were used. As controls, two containers having sterilized soil artificially inoculated with 25 g of inoculum and one container having sterilized soil plus 25 g of sterile oats-barley mixture were immersed in water to within 3 inches of their tops in each of the temperature tanks. The soil moisture was maintained at about 18 percent dry weight in all the containers. The experiment was repeated several times. Although the percentage of infection was often as high as that noted above, there were times when the inoculated plants were apparently almost free from the disease. The results of this experiment are summarized in table 4.

TABLE 4.—*Reaction of susceptible Dwarf Yellow milo to Pythium arrhenomanes in soil-temperature tanks at various temperatures in greenhouse at Manhattan, Kans., 1934–35*

Temperature (° C.)	Inoculated plants				Control plants (normal)
	Normal	Weak [1]	Dead	Total	
	Number	*Number*	*Number*	*Number*	*Number*
16	2	7	36	45	44
20	5	3	41	49	50
24	7	2	39	48	52
28	6	0	49	55	54
32	13	1	39	53	56
36	18	4	26	48	49

[1] Plants showing 1 or more leaves discolored or dead.

It is apparent that this particular *Pythium* isolate is pathogenic over a wide range of temperatures. At the higher temperatures there are more healthy or normal plants. This agrees with the results obtained in growing susceptible Dwarf Yellow milo plants in naturally infested soils in soil-temperature tanks thermostatically regulated to maintain fairly constant temperatures. The metal containers were filled with infested soil and moisture was added as needed. No attempt was made to vary the soil moisture in connection with the range of temperature used. The soil was kept at a moisture content of about 18 to 20 percent of its dry weight, as this was found satisfactory for the growth of the sorghum plants. The results of the studies in 1934 and 1935 at Manhattan are given in table 5. The growth of resistant and susceptible strains of milo in infested soil is compared at temperatures ranging from 16° to 36° C.

TABLE 5.—*Effect of soil temperatures on the development of the milo disease in susceptible and resistant strains of Dwarf Yellow milo when grown in Garden City infested soil in soil-temperature tanks in the greenhouse at Manhattan, Kans., 1934 and 1935*

Year and series	Soil temperature [1]	Susceptible plants			Resistant plants			Average height of plants	
		Living	Dead	Diseased and dead	Living	Dead	Diseased	Susceptible	Resistant
1934:	° C.	Number	Number	Percent	Number	Number	Percent	Cm	Cm
Series 1	16	[2] 1	24	100	27	0	0		
	20	[2] 3	18	100	23	0	0		
	23	[2] 3	20	100	19	0	0		
	26	[2] 2	14	100	16	0	0		
	29	[2] 1	22	100	23	0	0		
	34	9	9	50	22	0	0		
Series 2	16	[2] 4	25	100	35	0	0	8	21
	20	0	18	100	15	0	0	8	40
	23	[2] 3	28	100	29	0	0	10	43
	26	[2] 1	24	100	19	0	0	28	48
	29	[2] 1	26	100	23	0	0	15	50
	34	36	33	47.8	67	0	0	23	51
1935 [3]	16	[2] 2	17	100	14	0	0	9	24
	16	[2] 1	20	100	14	0	0	8	20
	20	0	16	100	19	0	0	12	31
	20	0	23	100	18	0	0	11	29
	24	0	20	100	14	0	0	9	34
	24	0	23	100	13	0	0	9	34
	28	0	25	100	20	0	0	10	37
	28	[2] 1	22	100	18	0	0	9	39
	32	[2] 1	18	100	17	0	0	13	39
	32	[2] 2	21	100	18	0	0	12	39
	36	9	13	59.1	22	0	0	28	40
	36	11	12	52.2	17	0	0	29	39

[1] The temperature range fluctuated ±1°–2°.
[2] 1 to 4 plants at these temperatures were diseased but not dead when final notes were taken.
[3] In 1935, 2 jars of plants were grown at each temperature range. The results from each jar are shown separately.

From the data in table 5 the following conclusions may be deduced: (1) Dwarf Yellow milo remains susceptible and plants may become infected at all soil temperatures used in these experiments. (2) The organism seems better able to infect at the lower than at the higher (30° to 36° C.) soil temperatures. (3) The resistance of the resistant selection of Dwarf Yellow milo is not broken down or altered under the soil temperatures and soil moisture used in these experiments. (4) Temperatures of 34° and 36° allow a considerable number of plants of susceptible Dwarf Yellow milo to escape lethal infection. If infection occurs at these temperatures, it is not sufficiently severe to kill all the plants. (5) Marked differences were observed in the height of susceptible and resistant Dwarf Yellow milo plants at the various temperatures used in these studies. This is due chiefly to the fact that in the susceptible Dwarf Yellow milo all the plants were dead when the height notes were taken, except in the 34° and 36° tanks.

Similar results were obtained in constant-temperature tanks at 20°, 25°, and 30° C. at Arlington Farm, where three susceptible varieties were compared with one resistant variety in noninfested as well as in infested soil. The resistant Fargo milo did not become infected in either clean or infested soil at any of the three temperatures. The disease symptoms developed more rapidly in all three susceptible varieties at 25°, but the disease was about equally severe at 20° and 25°. At 30° some of the plants escaped the disease.

Field Tests

Field inoculations with pure cultures of *Pythium arrhenomanes* were made during the period 1932–35 at Garden City and Manhattan and at Arlington Farm. Most of the inoculations were made by mixing the inoculum with the soil about the seed at planting time. In two inoculations the inoculum was mixed with the soil at one side of rows of plants 6 inches and 12 inches tall. The roots of these plants were injured. The cultures used for inoculation were grown on corn meal-carrot-humic acid agar, corn meal-sand medium, or oat-barley medium. Characteristic symptoms of the milo disease did not develop in above-ground parts of the inoculated plants in any of these experiments. When the plants were sectioned and examined at the end of the season they showed some reddening of roots and crown but no more in inoculated than in control plants.

At Garden City, in 1933, plots that had been inoculated the previous season were spaded and replanted, but no delayed infection or cumulative effect was apparent.

Experiments in the greenhouse have repeatedly shown that milo plants recover from the disease even when in pots of infested or inoculated soil, if able to grow through into uninfested soil in the benches. Since earlier inoculations in the field had been made by mixing cultures with the soil around and a few inches below the seed, the field inoculations in 1935 were made to a depth of 2 feet. The soil in plots 2 feet long, 2 feet wide, and 2 feet deep was taken out in 3-inch layers and replaced in the same order in which the layers came out. One plot was inoculated with a pure culture of *Pythium arrhenomanes*, another with diseased soil, and a third was left uninoculated. Dwarf Yellow milo was planted in 14-foot rows running through the center of each plot, making 6-foot extensions on either side on untreated ground. Four untreated 14-foot check rows were planted between these plots. Dwarf Yellow milo was planted June 10. While the plants grew normally above ground and all produced heads, examination of roots and crowns at the end of the season showed numbers of plants with reddened roots and crowns. *P. arrhenomanes* was reisolated from the roots. The results are given in table 6.

Table 6.—*Results of testing Dwarf Yellow milo in the field at Garden City, Kans., 1935, in infested soil and with pure cultures of Pythium arrhenomanes mixed with noninfested soil to a depth of 2 feet*

Treatment	Total plants	Diseased plants	
	Number	*Number*	*Percent*
Inoculated with *Pythium* cultures	32	25	78.1
Inoculated with infested soil	34	32	94.1
None (noninfested soil)	27	5	18.5

Vertical Distribution in the Soil

To obtain some idea of the vertical distribution of *Pythium* in infested areas at Garden City, soil was carefully removed in 3-inch layers to a depth of 24 inches. Before the removal of each 3-inch layer the soil was removed from around the four sides and wet cloths were laid down to prevent contamination of the lower layers by soil from nearer the surface. The soil from each layer was shipped in a tight container and was potted and planted to Dwarf Yellow milo

in the greenhouse at Arlington Farm. The results of these plantings are given in table 7.

While the number of plants is small, the data given indicate that infestation was greatest in the top 4 inches of soil and least in the soil 22 to 24 inches down. It is also evident that plants growing in the upper 12 inches of soil were all badly diseased, while those in the second foot of soil were only slightly diseased. All plants showed typical symptoms of yellowing and drying leaf tips. Check plants in sterilized Garden City soil showed no leaf discoloration or other symptoms of the milo disease. Plants grown in the top 3 inches of soil were larger than those grown in the next 3 inches, possibly owing to the effects of sunlight and drying on the amount of inoculum in the top soil. It is apparent that *Pythium arrhenomanes* occurs to some extent to a depth of 24 inches in the soil.

TABLE 7.—*Results of planting Dwarf Yellow milo, in Garden City infested soil removed in 3-inch layers to a depth of 24 inches to determine depth of soil infestation, in greenhouse at Arlington Experiment Farm, June 14, 1935*

Pot no.	Field depth of soil	Seeds		Condition of plants July 30			
		Planted	Germinated	Average height	Diseased	Dead	Red in crown
	Inches	*Number*	*Number*	*Inches*	*Number*	*Number*	
1	1-3	10	8	7	4	4	Dark red.
2		10	7	8	6	1	Do.
3	4-6	10	9	4	4	5	Do.
4		10	9	4	8	1	Do.
5	7-9	10	7	6	6	1	Red.
6		10	8	8	6	2	Do.
7	10-12	10	9	7	8	1	Do.
8		10	9	7	7	2	Do.
9	13-15	10	9	10	8	1	Trace of red.
10		10	9	10	9	0	Do.
11	16-18	10	10	9	10	0	No red.
12		10	10	9	9	1	Do.
13	19-21	10	9	9	6	3	Trace of red.
14		10	8	10	8	0	Do.
15	22-24	10	10	10	10	0	Trace of red in 3.
16		10	9	12	9	0	No red.

LONGEVITY IN THE SOIL

Experiments have shown that infested soil carried over in the greenhouse for 4 years is capable of producing 100-percent infection when replanted to susceptible milo. In the field, infested soil cropped to a resistant sorghum for 1 year and then planted to Dwarf Yellow milo will cause the disease. Observations by the writers and studies by Wagner (*35*) show conclusively that 1 year's fallowing has no effect in eliminating the infective capacity of contaminated soil. Also, if milo is grown on previously infested land that has been fallowed for 1 year, then sown to wheat for 1 or 2 years, followed by a year of fallow before planting to milo, the milo disease will again become prevalent. This shows that under field conditions the causal organism remains in the soil for at least 4 years.

HOSTS

Isolations of *Pythium arrhenomanes* from milo have been tested for pathogenicity on corn and sugarcane in the greenhouse at Arlington Farm.

When cultures of *Pythium arrhenomanes* from milo were stirred into the soil about the roots of young seedling plants of Golden Bantam sweet corn (Inbred 8482–3–1)[8] the inoculated plants turned light

[8] Seed furnished by G. M. Smith, Purdue University Agricultural Experiment Station, La Fayette, Ind.

yellow in color and at the end of 2 months were only one-half to two-thirds the size of uninoculated plants. The results of growing yellow dent field corn (Inbred A48)[9] in steamed Garden City soil, in steamed soil to which *P. arrhenomanes* had been added, and in infested Garden

FIGURE 12.—Yellow dent corn (Inbred A48, Holbert) planted and inoculated May 3, 1935, at Arlington Experiment Farm: *A*, Grown in naturally infested Garden City soil steamed 4 hours at 100° C.; *B*, grown in naturally infested Garden City soil steamed 4 hours at 100° and inoculated with a 3-day corn-meal agar culture of *Pythium arrhenomanes* at planting time; *C*, grown in naturally infested Garden City soil. Photographed May 31, 1935.

City soil are shown in figure 12. Plants in steamed soil grew normally; those in infested soil made less growth and at the end of 4 weeks were less than half the size of the plants in steamed soil. The leaves turned yellow and dried, and examination of the roots showed that

[9] Seed furnished by J. R. Holbert, Bloomington, Ill.

all of the finer roots had been destroyed. Plants in the soil to which *P. arrhenomanes* had been added also were stunted, were about half the size of the checks, and showed the same disease symptoms as corn plants in naturally infested soil.

Growth of sugarcane (Louisiana Purple) in steamed Garden City soil, in steamed soil inoculated with *Pythium arrhenomanes*, and in

FIGURE 13.—Louisiana Purple sugarcane repotted and inoculated May 3, 1935, at Arlington Experiment Farm: *A*, Grown in naturally infested Garden City soil steamed 4 hours at 100° C.; *B*, grown in naturally infested Garden City soil steamed 4 hours at 100° and inoculated with a 3-day corn-meal agar culture (Dwarf Yellow milo 3) at potting time; *C*, grown in naturally infested Garden City soil. Photographed May 31, 1935.

infested Garden City soil gave similar but less striking results. Plants from the three soils are shown in figure 13. The leaves of plants in infested soil and in soil to which *P. arrhenomanes* had been added droop and have the appearance of having been affected by drought. Much of the root system was destroyed by *Pythium*.

VARIETAL RESISTANCE AND SUSCEPTIBILITY

FIELD TESTS

A striking feature of the milo disease is the manner in which different varieties of sorghum vary in susceptibility (*35*). Certain strains of Dwarf Yellow milo (C. I. 332), Double Dwarf Yellow milo (C. I. 868), Early Yellow milo (T. S.[10] 21195), Early White milo (C. I. 480), Day milo (C. I. 959), Sooner milo (C. I. 917), Pygmy milo (C. I. 1010), Wheatland (C. I. 918), Beaver (C. I. 871), Desert Bishop (C. I. 870), darso (C. I. 615), and certain hybrids one parent of which is either milo or darso have been found to be very susceptible to the milo disease. Since milo has been used as a parent in most crosses to produce high-yielding grain sorghums that can be harvested with the combine, most combine varieties are very susceptible. In contrast with this, the kafirs, sorgos, feteritas, and other sorghums grown in infested soil were found to be resistant.

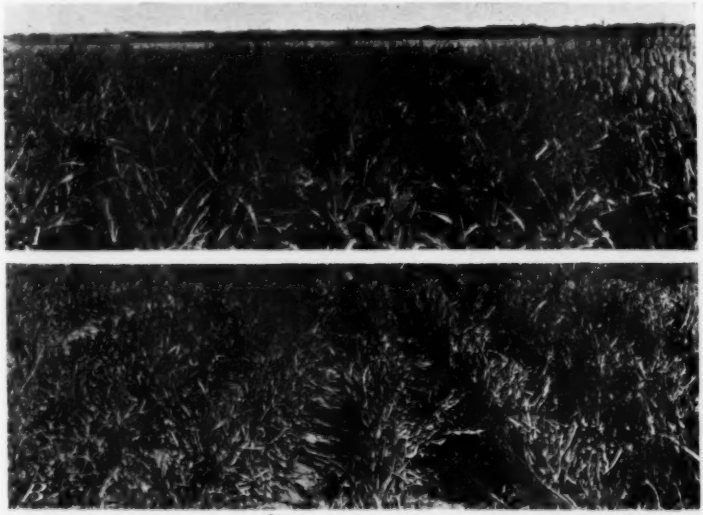

FIGURE 14.—Susceptible Dwarf Yellow milo grown on the agricultural experiment station at Fort Hays, Kans. *A*, On noninfested soil; *B*, on infested soil. This field was completely destroyed; the plants were killed before they produced heads. These photographs show the seriousness of the disease under farm conditions.

Wagner (*35*) has classed the various sorghums as susceptible, resistant, immune, and segregating from data obtained over a 5-year period from plants grown in the field on naturally infested soil at Garden City. Table 8 gives the results of field plantings of some of the 76 varieties grown in infested soil at Garden City in 1931. Varietal differences remained the same in similar tests in subsequent years, but in these later years the disease was more severe and susceptible varieties produced no grain at all. The seriousness of the disease under farm conditions is shown in figure 14.

[10] T. S. refers to Texas station number.

TABLE 8.—*Reaction of sorghum varieties to the milo disease at the Garden City substation of the Kansas Agricultural Experiment Station, 1931*

[All varieties were planted June 12 and emerged June 16 to 18]

Source of seed	Record no.	Variety	Date disease was first apparent	Disease reaction [1]	Remarks
Garden City, Kans.	C. I. 332	Dwarf Yellow milo [2]	Aug. 3	HS	No grain.
Do	C. I. 332	Dwarf Yellow milo	July 17	HS	Do.
Do	G. C. C. 11 [3]	Dwarf Yellow milo selection.	July 27	R	Plants normal. Excellent.[4]
Do	G. C. C. 21	do	do	Seg	13 diseased and 3 healthy milo plants. Row has 1 healthy and 1 diseased hybrid, also.
Hays, Kans.	C. I. 480	Early White milo [2]	July 17	HS	No grain.
Garden City		Hybrid (Early White milo).	do	Seg	2 plants diseased, others healthy.
Woodward, Okla.	C. I. 918	Wheatland [2]	do	HS	No grain.
Garden City	G. C. 301	Wheatland hybrid	do	Seg	11 normal and 15 diseased plants. Many types of plants.
Manhattan, Kans.	Man. 30-110	White darso [2]	do	HS	Plants stunted but produced small heads.
Woodward	C. I. 871	Beaver	July 21	HS	No grain.
Do	Wdw. 8-2-6	Kafir×Dwarf Yellow milo [2].	June 30	R	Produced normally.[4]
Do		Durra×Dwarf Yellow milo.	July 17	HS	Very poor grain. 1 plant small but apparently healthy, others dead.
Do	C. I. 615	Darso	do	HS	No grain.
Garden City	G. C. 306	Custer selection (tight sheath).	do	HS	No grain.
Do	G. C. 301	Custer selection (loose sheath).	do	Seg	Trace of disease in some plants but most are practically normal. Row good. Excellent grain.
Hays	H. C. 305 [5]	White Custer	do	R	Normal. Good grain on all plants.
Manhattan	F. C. 6611 [6]	Early Sumac [2]	July 21	I	All plants normal.[4]
Do	F. C. 6610	Leoti Red [2]		I	Do.
Do	F. C. 7038	Red Amber	July 30	I	Do.
Do	Sel. 2473	Red Amber×Feterita		I	Do.
Do	C. I. 182	Feterita [2]		I	Do.
Woodward	C. I. 623	Spur feterita [2]		I	Do.
Do	C. I. 864	Dwarf feterita (Woodward No. 1).		I	Do.
Do	C. I. 812	Kaferita [2]		I	Do.
Garden City	C. I. 872	Wonder [2]		I	Do.
Hays	C. I. 620	Hegari		I	Do.
Woodward	C. I. 874	Chiltex [2]		I	Do.
Manhattan	C. I. 873	Premo [2]		I	Do.
Do	F. C. 6620	Ajax [2]		I	Do.
Woodward	F. C. 8951	(Feterita × kafir) × kafir.[2]		I	All plants normal.
Do	C. I. 814	Bishop [2]		R	Do.
Lubbock, Tex.	F. C. 8991	Manko maize		R	Do.
Hays	C. I. 902	Kalo [2]	July 21	I	Good grain.[4]
Do	C. I. 901	Club [2]		I	All plants normal.
Do	C. I. 904	Dawn selection [2]		I	Do.
Do	C. I. 906	Western Blackhull [2]		I	Do.
Manhattan	C. I. 473	Pink kafir [2]		I	Do.
Garden City	C. I. 472	Sunrise [2]		I	Do.
Woodward	C. I. 628	Reed kafir [2]	July 21	I	Do.[4]
Manhattan		Mobray	July 6	R	Do.[4]
Do	C. I. 920	Grohoma [2]	do	I	Do.[4]
Do	Fengel	Grohoma		I	All plants normal. Better type than C. I. 920.
Hays	C. I. 971	Dwarf Freed		I	All plants normal.
Manhattan	C. I. 905	Modoc [2]	July 3	I	Do.[4]
Tribune, Kans.	C. I. 972	Greeley		I	Do.
Garden City	C. I. 332	Dwarf Yellow milo [2]	July 21	HS	All plants diseased but some produced poor heads.

[1] HS=highly susceptible; S=susceptible; Seg=segregating; R=resistant; and I=immune.
[2] Self-pollinated.
[3] G. C. C. refers to Garden City cereal number.
[4] Showed orange coloring early, but no further evidence of the disease developed.
[5] H. C. refers to Hays cereal number.
[6] F. C. refers to accession number of Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

GREENHOUSE TESTS

Early in the studies on this disease it became evident that the reaction of a variety in the seedling stage in the greenhouse was a safe index of its reaction in the field. This fact is well established by the results of several years' work, as shown in table 9. Considerable time can be saved in testing the selections made in the field by first growing them in the greenhouse during the winter months. The resistant selections can be separated from the susceptible or segregating ones, and thus the number of selections can be reduced in the list for spring

FIGURE 15.—Sorghum selections grown in naturally infested Garden City soil in the greenhouse at Manhattan, Kans. *A*, Left rear, Club kafir, resistant; left front, Dwarf Yellow milo, susceptible; right, Day milo selection (Wagner), susceptible. *B*, Left rear, Club kafir, resistant; left front, Dwarf Yellow milo, susceptible; right, Wheatland selection (Wagner), resistant. Photographed April 19, 1934.

planting. This can best be shown by the following examples which represent a few of the selections tested. One of the writers (Wagner) made selections from plants of Day milo, Wheatland, and Beaver that seemed resistant in the field. These selections were tested in infested Garden City soil in the greenhouse at Manhattan during the fall and winter (fig. 15), and the results were used as a guide in making up the list for field planting the following spring at Garden City.

TABLE 9.—*Comparison of the reaction of selections of sorghums to pythium root rot in the greenhouse and field at Manhattan and Garden City, Kans., 1933 and 1934*

Year and packet no.	Variety or selection	C. I. no.	Total plants	Diseased plants in greenhouse		Reaction [1]	
						Greenhouse, Manhattan	Field, Garden City
1933			*Number*	*Number*	*Percent*		
7	Dwarf Yellow milo selection	332	12	0	0	R	R
41	Beaver selection	871	11	0	0	R	R
47	do	871	12	0	0	R	R
48	do	871	13	0	0	R	R
49	do	871	14	0	0	R	R
50	do	871	12	0	0	R	R
Control	Dwarf Yellow milo	332	62	62	100	S	S
Do	Beaver	871	50	50	100	S	S
1934							
1	Beaver selection	871	23	0	0	R	R
3	do	871	23	0	0	R	R
5	do	871	21	0	0	R	R
6	do	871	20	0	0	R	R
12	do	871	20	16	44.4	Seg.	Seg.
14	do	871	22	14	38.9	Seg.	Seg.
15	Dwarf Yellow milo selection	332	23	0	0	R	R
16	do	332	22	0	0	R	R
17	do	332	23	0	0	R	R
27	Wheatland selection	918	24	0	0	R	R
28	do	918	16	0	0	R	R
31	do	918	20	0	0	R	R
33	do	918	17	0	0	R	R
34	Day milo selection	959	22	2	8.3	Seg.	Seg.
35	do	959	22	7	24.1	Seg.	Seg.
36	do	959	18	12	40	Seg.	Seg.
37	do	959	22	0	0	R	R
38	do	959	20	20	100	S	S
39	do	959	24	24	100	S	S
Control	Dwarf Yellow milo	332	31	31	100	S	S
Do	Wheatland	918	30	30	100	S	S
Do	Beaver	871	35	35	100	S	S

[1] R=resistant; S=susceptible; Seg.=segregating.

INHERITANCE OF RESISTANCE

Studies of the inheritance of resistance to the milo disease, made by Bowman et al.[11] at Manhattan and Garden City and at Arlington Experiment Farm, show that susceptibility is partially dominant and due to a single genetic factor. They investigated progenies of the cross Dwarf Yellow milo (susceptible) × Club (resistant) (fig. 16). Resistant, segregating, and susceptible F_3 lines were secured. The data show that resistance is dependent upon the expression of a single recessive factor, *s*, carried by Club and resistant selections of Dwarf Yellow milo. No evidence was found for any complementary factors influencing disease susceptibility. Figure 17 shows the reaction to the milo disease of F_1 hybrids of resistant × susceptible parents when grown in infested soil.

[11] BOWMAN, D. H., MARTIN, J. H., MELCHERS, L. E., and PARKER, J. H. INHERITANCE OF RESISTANCE TO PHYTHIUM ROOT ROT IN SORGHUM. [In manuscript.]

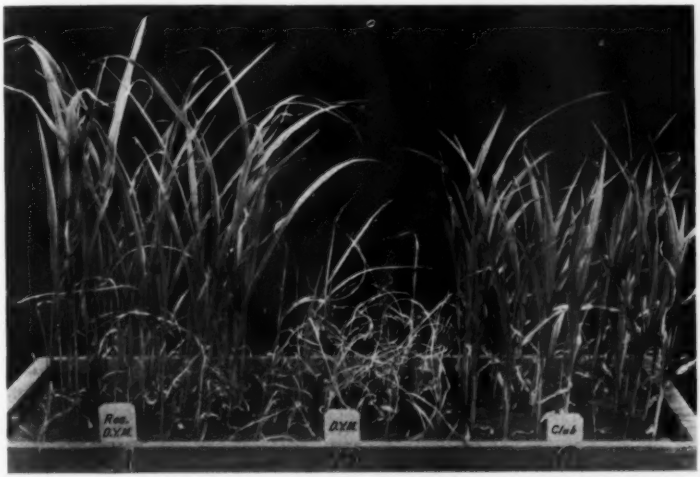

FIGURE 16.—Resistant and susceptible sorghums grown in naturally infested soil at Manhattan, Kans. *A*, Dwarf Yellow milo selection (Wagner), resistant; *B*, Dwarf Yellow milo, susceptible; *C*, Club, resistant. Photographed January 1934.

FIGURE 17.—Sorghum seedlings, grown at Manhattan, Kans., in naturally infested Garden City soil, showing inheritance of resistance to *Pythium arrhenomanes*. *A*, Feterita (C. I. 182), resistant parent; 10 plants, all healthy. *B*, F_1 feterita (C. I. 182)×Dwarf Yellow milo (C. I. 332); 28 plants, 7 resistant, 21 dead. *C*, F_1 feterita (C. I. 182) × Dwarf Yellow milo (C. I. 332); 31 plants, 9 resistant, 22 dead. *D*, Dwarf Yellow milo (C. I. 332), susceptible parent, 12 plants, all dead. Photographed May 22, 1933.

CONTROL

The soil on the station at Garden City, Kans., is a fine silt loam slightly alkaline in reaction. Soil analyses in 1931 by the Bureau of Chemistry and Soils, United States Department of Agriculture, showed no chlorides or sulphates in either infested or clean soil and no excess of nitrates. The percentage of total soluble salts was 0.125, indicating that the trouble at Garden City was not due to any excess of soluble salts. The results of colorimetric and electrometric tests of the reaction of this soil and of soils from other localities were as follows. Quinhydrone electrode [12] (mud ball) tests of Garden City soil: Noninfested pH 6.18, infested pH 7.41. Colorimetric (La Motte) tests of soil: From Garden City, Kans., noninfested pH 7.6, infested pH 8.0; from Dalhart, Tex., noninfested and infested, pH 7.2–7.8; from Robstown, Tex., noninfested and infested pH 7.6–8.0; from Chillicothe, Tex., pH 6.8–7.0. The colorimetric values are averages of 10 determinations.

Since the infested soils were alkaline in reaction, sulphur was used in three experiments to reduce the alkalinity in order to determine the effects of change of reaction on the disease-carrying power of the soil.[13]

Flowers of sulphur at the rate of 1,000 pounds and 500 pounds per acre; aluminum sulphate, 500 pounds and 250 pounds per acre; ammonium sulphate plus ferrous sulphate, 250 pounds per acre, were added to the soil 1 month before planting. While flowers of sulphur changed the soil reaction in the greenhouse to pH 5.6 and 6.4 as compared with pH 7.8 in untreated infested soil, this change had no apparent effect in reducing the amount of disease. Myers (*22*), in 1934, reported similar results, stating that "sulphur and sulphuric acid had no beneficial effect" in reducing the disease on infested Garden City soil in pots in the greenhouse.

Early in these investigations it was found that steaming the soil at 15 pounds pressure for 1 hour removed the cause of the disease (fig. 18). Subsequent soil treatments in the greenhouse at Manhattan, at Arlington Farm, and in the field at Garden City showed that formalin (fig. 19) (1 part commercial in 49 parts of water, or 120 cc of formalin in 880 cc of water) and also acetic acid (fig. 20) (glacial acetic 5 cc in 495 cc of water) were effective in destroying the causal organism. In two plots, each 4 by 6 feet, on infested soil in the field, the soil was removed in 2-inch layers to a depth of 12 inches. One plot was treated with formalin and the other with water, and the layers of soil were then replaced in the order in which they were removed. Plants in the formalin-treated plot grew normally and produced good heads, while plants in the water-control plot and in the infested soil outside the formalin-treated plot all developed typical symptoms of the milo disease. When the formalin-treated field plots at Garden City were replanted the second season without further treatment, the effects of the formalin treatment had practically disappeared; plants in treated and in untreated areas were equally diseased.

[12] Made by the Bureau of Chemistry and Soils, U. S. Department of Agriculture.

[13] The senior author is indebted to F. R. Reed, of the Bureau of Chemistry and Soils, U. S. Department of Agriculture, for assistance in soil studies.

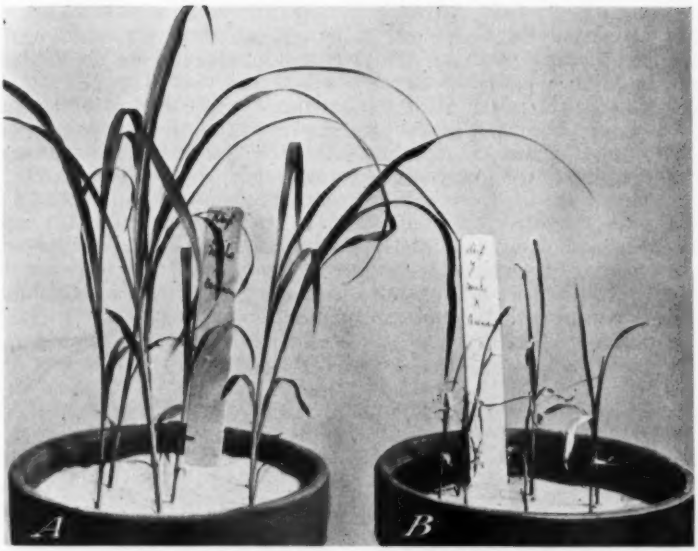

FIGURE 18.—Susceptible Dwarf Yellow milo (C. I. 332) planted in naturally infested Garden City soil at Arlington Experiment Farm: *A*, Soil steamed for 1 hour at 15 pounds pressure; *B*, untreated soil. Planted December 23, 1930; photographed January 24, 1931.

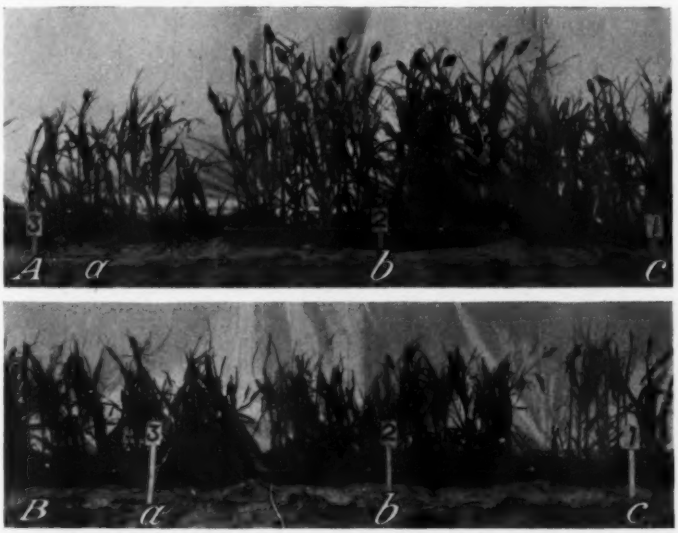

FIGURE 19.—Results of formalin treatments of naturally infested soil on Dwarf Yellow milo plants grown in the field at Garden City, Kans., 1931. *A*, *a*, and *c*, untreated areas; *b*, soil treated with formaldehyde solution (1 part 40-percent formalin to 49 parts of water). *B*, *a* and *c*, Untreated areas; *b*, water control (soil removed and replaced in 2-inch layers as in the formalin-treated box but saturated with water instead of formalin solution).

Myers (*22*), in 1934, used calcium nitrate, sodium nitrate, ammonium nitrate, superphosphate, potassium chloride, ammonium sulphate, magnesium sulphate, manganous sulphate, and copper sulphate on infested Garden City soil. While nitrogen tended to prolong the life of the plants, none of the treatments was effective in preventing the disease. His results confirm those obtained by the writers, namely, that neither the alkaline soil reaction, nor the soil nutrients, nor amendments are responsible for the development of the disease.

The effect of nitrogen as a factor in predisposing milo plants to disease was studied at Manhattan in 1935. Carpenter (*5*) cites experiments showing that abnormal nutrition predisposes sugarcane plants to root rot caused by *Pythium*. He believes that resistance of certain varieties of cane is changed to varying degrees of susceptibility by modifying the nutritional environment and that nitrogen is one of the modifying elements. In the studies at Manhattan, ammonium sulphate was added to infested soil in jars at the rate of 300, 700, and

FIGURE 20.—Results of acetic acid treatments on Dwarf Yellow milo plants grown in naturally infested Garden City soil at Arlington Experiment Farm: *A*, *B*, and *C*, Untreated soil; *D*, *E*, and *F*, soil treated with glacial acetic acid (1:100). Treated March 15; planted April 4; photographed May 16, 1932.

1,000 pounds per acre. The jars were planted so that susceptible Dwarf Yellow milo, a resistant selection of Dwarf Yellow milo, and Club (resistant) were growing side by side in each jar. The results of several tests show (1) that nitrogen in these quantities does not predispose the resistant selections of Dwarf Yellow milo to the disease, and (2) that the susceptible Dwarf Yellow milo remains susceptible, although the life of the plants is prolonged somewhat by addition of nitrogen. Vegetative growth occurs in susceptible Dwarf Yellow milo in proportion to the rate of nitrogen applied, i. e., the larger amounts of nitrogen produce the most growth, but the plants do not escape infection. Nitrogen applications caused marked increase in growth in the resistant selections of Dwarf Yellow milo and Club.

Fellows (*18*), in studies on *Ophiobolus graminis*, showed that additions of chicken manure to infested soils will control the take-all disease of wheat. It appeared of interest, therefore, to see what

effect the addition of various quantities of chicken manure to infested soil would have on the milo disease.

In 1933, four lots of Garden City infested soil were mixed with 3.4, 3.1, 2.7, and 2.4 percent by volume of dried, ground chicken manure. Plants of susceptible Dwarf Yellow milo and resistant Club were grown in each jar. These quantities of chicken manure prevented the normal growth of both the milo and Club seedlings. When the quantity of chicken manure was reduced to a point that allowed the sorghum plant to grow, the disease was not reduced in the slightest degree.

Fallowing and crop rotations have shown that the causal organism remains alive for 4 years and that it can produce infection in susceptible sorghums during that period.

Studies have shown that the development of resistant strains or varieties is the only feasible method of control for the milo disease. Resistant selections of Dwarf Yellow milo, Beaver, Day milo, and Wheatland have been made. These selections have maintained their resistance during several years' test (fig. 21). They can be planted on

FIGURE 21.—Susceptible Dwarf Yellow milo and the resistant selection of Dwarf Yellow milo made by Wagner, grown in naturally infested soil at Garden City, Kans., 1933: *a* and *d*, Dwarf Yellow milo selection, resistant; *b* and *c*, Dwarf Yellow milo, susceptible.

infested soil where ordinary Dwarf Yellow milo, Beaver, Day milo, and Wheatland are a complete loss. While the resistant selections of Beaver, Day milo, and Wheat land have been made more recently than those of the resistant Dwarf Yellow milo, they have been tested in the greenhouse and in the field and apparently are as resistant as the resistant Dwarf Yellow milo selection (fig. 22). Seed of the resistant Dwarf Yellow milo selection is not yet available for general distribution.

Fertilizers as a means of stimulating growth and so reducing infection were tested at Arlington Farm in 1931 on infested and clean soils from Garden City, Kans., and from Robstown and Chillicothe, Tex.

Three pots of each soil received no treatment and served as checks. Twelve others received the following treatments: 3 plots received sodium nitrate ($NaNO_3$) at the rate of 200 pounds per acre; 3 plots received superphosphate at the rate of 300 pounds per acre; 3 plots received potash (KSO) at the rate of 100 pounds per acre; 3 plots received complete fertilizer 5–8–5 at the rate of 400 pounds per acre.

These fertilizers apparently had no effect in altering the amount of disease in the Garden City and Robstown infested soils. Plants in treated and untreated infested soils grew to 6 inches in height, disease symptoms developed, and the plants died. In all three Chillicothe

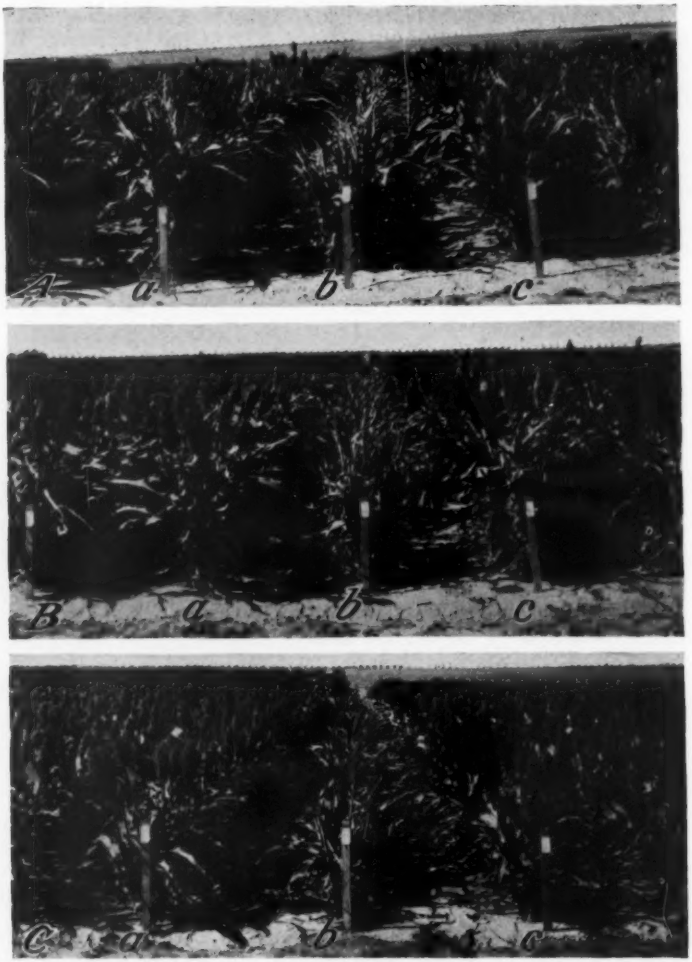

FIGURE 22.—Susceptible milos grown between resistant selections of the same varieties. *A*, Beaver milo: *a* and *c*, Resistant selections; *b*, susceptible. *B*, Wheatland milo: *a* and *c*, Resistant selections; *b*, susceptible. *C*, Day milo: *a* and *c*, Resistant selections; *b*, susceptible.

soils (infested and treated, infested and untreated, and noninfested) the plants were vigorous and healthy and continued so to maturity. The only sign of disease in plants in this soil was a slight reddening in the crowns of a few plants.

DISCUSSION

Comparative studies of the so-called milo disease in the Southwest have shown that more than one type of injury occurs. At Garden City, Kans., the disease is a typical root rot that retards the growth of the plants. When the root rot is severe, milo plants turn yellow and die without heading. The reddening of roots and crown is a host reaction to injury by a species of *Pythium* and possibly secondary invaders. This type of injury, resulting from rotting of the finer roots, is caused by *P. arrhenomanes* and occurs in widely separated localities in Kansas, Texas, Oklahoma, New Mexico, and probably in other States. Soil sterilization with formalin, steam, and acetic acid solutions, destroys the *Pythium* so that susceptible milos develop normally. Resistant selections of milo have been selected that develop normally and produce seed on infested soil where susceptible milos die without heading.

At Chillicothe, Tex., a somewhat similar stunting of milo plants has occurred which has also been called the milo disease. At Chillicothe, however, there is little or no root rot or accompanying red discoloration of roots and crown. In addition, the injured plants at Chillicothe tiller abundantly, and in many cases there is rotting of the central shoot. At Chillicothe, soil sterilization did not prevent development of the disease, and both resistant and susceptible strains of milo developed some disease symptoms and some good heads. These facts and the abundance of chinch bugs (*23*) at Chillicothe indicate that the milo injury at this station may differ both in cause and symptoms from the milo disease as it occurs in other parts of Texas and at Garden City.

Pythium arrhenomanes may possibly occur to some extent in the soils at Chillicothe, and it is very possible that the pythium root rot will eventually occur in wider areas than known at present. The fact that this species of *Pythium* causes root rots in Canada and in the central and southern United States, as well as in other parts of the world, indicates a wide distribution. Why it causes severe root rot in certain areas and not in others is not clear. The growing of susceptible varieties of milo year after year in the same plot may lead to a quantitative increase sufficient to produce disease symptoms, or possibly a qualitative change in the virulence of the fungus may be the cause of the serious root rots.

Fusarium moniliforme can readily be isolated from diseased milo roots and it is probably for this reason that the disease in some sections has been attributed to this fungus. The writers have not been able to demonstrate in any of their inoculation experiments that *F. moniliforme* is in any way responsible for the development of the root rot of milo. Numerous isolations of this species have been used repeatedly in pure-culture inoculations and also in combination with cultures of *Pythium arrhenomanes*. In pure culture they have not rotted the roots, and in combination with *P. arrhenomanes* the results have been the same as when pure cultures of the latter fungus alone were used.

There has recently arisen some question as to the proper species name for the *Pythium* causing root rots of corn, wheat, and sugarcane. Rands and Dopp (*26*), in 1934, included the isolations from all of these hosts in the one species *P. arrhenomanes*. Carpenter (*7*), in 1934, stated that *P. arrhenomanes* and *P. graminicolum* Subramanian are identical and that, because of priority of publication, *P. graminicolum* should take precedence over *P. arrhenomanes*. Drechsler (*10*) has

recently compared these two species and still regards them as separate species. The *Pythium* isolated from milo has been identified as *P. arrhenomanes.*

While *Pythium arrhenomanes* has proved virulently pathogenic on susceptible milos in the greenhouse, inoculations in the field have not produced typical infection. Similar difficulties have been encountered by others in obtaining positive results from field inoculations on other graminaceous hosts of *P. arrhenomanes* and also in work on other soil-borne diseases (*2, 14*). Antagonism of other soil organisms offers a possible explanation for these difficulties. In most of the field inoculations with *P. arrhenomanes,* the inoculum was mixed with the upper few inches of soil about the seed. Small quantities of infested soil used for inoculation in the field in this same way did not produce disease symptoms. It is probable that, as in some instances in the greenhouse, the roots were able to grow out into uninoculated or non-infested soil and to escape or outgrow the disease. The results of the field inoculations conducted in 1935 tend to bear out this suggestion and to offer some encouragement for further field inoculations. Infection was not severe enough to produce symptoms above ground, but infections on roots occurred on only 18 percent of the uninoculated plants as compared with 78 and 94 percent of plants inoculated with *P. arrhenomanes* and with infested soil.

Pythium root rot of milo first attracted attention in small experimental plots in Kansas and Texas. Since that time it has been found in commercial fields in several counties in Texas and has been severe enough to prevent the growing of milo in the infested fields. As reports of diseased milo and specimens of diseased plants from other localities continue to be received, it seems probable that the disease is more widely distributed than present knowledge indicates. The selection of resistant plants of susceptible varieties made by one of the writers (Wagner), the testing of these, and their increase for distribution will soon make it possible to offer resistant strains that will grow in areas where root rot would otherwise be a serious limiting factor in milo production.

SUMMARY

During the last 10 years a root rot of milo has become increasingly destructive in Kansas, Texas, and New Mexico.

The plants are stunted or killed and the yield is much reduced. The finer roots are destroyed, while the larger roots and interior of the crown become dark red to purplish red in color. Diseased plants have the general appearance of having fired.

Pythium arrhenomanes has been isolated from the diseased roots and has been shown by inoculations in the greenhouse to be actively parasitic on milo roots.

Pythium arrhenomanes from milo also attacks sugarcane and yellow dent corn.

Milos, certain milo crosses, and darso are very susceptible to the disease, while kafirs, sorgos, feteritas, and some other sorghums are resistant.

Soil amendments and fertilizers do not prevent milo plants from becoming diseased when grown in infested soil.

The disease is readily transmitted by mixing small quantities of infested soil or infected root and crown tissue with noninfested soil

or by soil-water leachings from infested soils, indicating that these are ways in which it is spread under natural conditions. The disease is not transmitted by the use of water leachings of infested soil which have passed through a Berkefeld filter. This indicates that the organism is excluded and that the causal factor is not a soil-borne virus.

Soils on which a diseased crop has been grown remain infested with the organism over a period of at least 4 years. Ordinary rotations or fallow have not controlled the disease.

Although soil sterilization by means of steam, formaldehyde, and acetic acid is effective, it would not lend itself to field practice.

The development of a resistant strain of Dwarf Yellow milo has been accomplished, and resistant strains of Beaver, Wheatland, and Day milo are now being tested at Garden City.

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MORPHOLOGICAL CHARACTERS OF TEOSINTE CHROMOSOMES [1]

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INTRODUCTION

The study of the morphology of chromosomes might be said to have begun soon after their discovery 50 years ago. In spite of their minute size it was soon recognized that they differ in size and that the relative size of each chromosome of a set is constant in the different tissues of an animal or plant. As early as 1900 it had been observed that the chromosomes of a somatic cell were in pairs of equal length. These pairs are now termed homologues. The size and appearance of the chromosome at metaphase of both mitotic and meiotic divisions soon received attention, and many investigators were able to differentiate each individual chromosome of certain plants and animals. Their illustrations showed not only length differences but also characteristic differences in the shapes of the chromosomes. It soon was recognized that the differences in the shapes of chromosomes on the division spindle were due to the position at which the spindle fiber was attached to the chromosome. A median point of attachment results in bending a chromosome into the shape of a V, a subterminal point of attachment bending the chromosome into the shape of a J, and a terminal point of attachment leaving the chromosome unbent.

The extended thread stage or prophase of the first reduction division in maize (*Zea mays* L.) and its relatives has been found by recent investigators to be superior to the more contracted metaphase chromosomes for the study of morphological characters. In this tribe of grasses, not only are differences in length more pronounced in the earlier phases and the region of spindle-fiber attachment more clearly shown, but certain deeply staining enlarged regions, known as knobs, are found. These knobs occur at certain definite points along the threads of many of the chromosomes and, together with the other morphological characters, permit the unquestionable identification of chromosomes.

Chromosome length and the position of the spindle-fiber attachment region and knobs are morphological features of importance in tracing the relationship between maize and its relatives.

The pioneer studies of McClintock (*7*, *8*)[2] demonstrated the presence of knobs on all but 1 of the 10 chromosomes of corn. More recently Beadle (*2*) has demonstrated similar knobs terminating many of the chromosomes of Florida teosinte.

The writer has attempted a critical examination of the morphological characters of the chromosomes of all the types of teosinte available,

[1] Received for publication Aug. 22, 1936; issued June, 1937.
[2] Reference is made by number (italic) to Literature Cited, p. 862.

hoping that a comparison of their chromosome structure would throw additional light on the relationship of the various teosintes to each other and to maize.

MATERIAL

Perennial teosinte (*Euchlaena perennis* Hitchc.) from Jalisco, Mexico, called throughout the text "perennial," and annual teosinte (*Euchlaena mexicana* Schrad.) from seven different sources were available for study. One of the annual strains, designated Florida, was from the commercial stock grown in Florida. Four strains came from Guatemala and two from Mexico. Two of the Guatemalan strains were collected by the Popenoes (*4*) in southern Guatemala in the Department of Jutiapa near the towns of El Progreso and Moyuta, and have been designated by the place names Progreso and Moyuta. The other two strains were collected by Popenoe and Kempton while on an expedition for the Carnegie Institution of Washington and the United States Department of Agriculture and have been designated Nojoyá and San Antonio from the localities where the material was collected—Nojoyá and San Antonio Huixta, in the Department of Huehuetenango, northern Guatemala. Three separate lots of seed of a Mexican teosinte were furnished by Walter Douglas from the Mexican Agricultural Station at Chapingo, near Mexico City. These are considered together under the name Chapingo. Another lot of seed, called Durango, came originally from Durango, Mexico. However the seed for the plants used in this study was supplied by R. A. Emerson, of Cornell University, and may represent several generations of inbreeding.

With the exception of the cytological material from the two teosinte strains recently collected by Popenoe and Kempton, the material used in this study was from progenies of perennial and annual teosintes, grown at Coconut Grove, Fla., Torrey Pines, Calif., at the Arlington Experiment Farm, Arlington, Va., or in the greenhouses of the United States Department of Agriculture at Washington, D. C. Cytological collections were made at as many of these localities as possible, to insure against the material being unsatisfactory, as is frequently the case if plants are grown in unfavorable environments. The cytological material from Nojoyá and San Antonio was brought to the Washington laboratory by Kempton and consisted of a few collections made from plants growing in their natural habitat.

METHODS

Young tassel branches were killed immediately with acetic-absolute alcohol (1:3). After 12 hours, although sometimes not until the material reached the laboratory at Washington, D. C., the killing fluid was removed and replaced with absolute alcohol. The specimens were corked tightly and left until used for study. Aceto-carmine smear preparations were found satisfactory for studying the midprophase, or the extended thread stage of the pollen mother cells. The slides, after being thoroughly examined, were preserved by a modification of McClintock's method (*6*) as permanent mounts. The cover glasses were soaked from the slides, and both the cover glasses and slides were dehydrated in 1:3 acetic-absolute alcohol, cleared, and remounted with Canada balsam, a procedure that usually preserved the preparations satisfactorily.

Examination of the thread stages was made with 2-mm and 1.5-mm objectives and×7.5 and×15 oculars. Camera-lucida drawings were made of individual chromosomes, of groups of chromosomes, and, as frequently as possible, of cells in which all 10 paired chromosomes could be traced. These drawings were used to determine the length, the number and position of the knobs, and the position of the spindle-fiber attachment of each chromosome.

The drawings illustrating this article show the characteristic markings of the chromosomes in their observed positions. The chromomeres along the associated threads are, however, shown only in a general manner, their number and positions being represented only approximately. Counts of the number of chromomeres on a chromosome or on some portion of a chromosome indicate that, on an average, they are about a micron apart. The pair-by-pair association of chromomeres is not always apparent. Frequently sections of the associated chromosomes, well-stained for critical examination, show the chromomeres alternating and not opposite, as would be expected in a chromomere-to-chromomere association.

The length of the chromosomes and the distance of the knobs and of the spindle-fiber attachment region from the nearer end of the chromosome were determined by measuring with a map rule the camera-lucida drawings of each chromosome. These measurements, based on a drawing in one plane, are not strictly accurate when they represent a chromosome varying in depth of focus, nor do they take into account the possibility that all chromosomes or parts of chromosomes may not be in the same stage of expansion or contraction. Frequently the chromosomes of the same cell or portions of the same chromosome do not seem to contract simultaneously. In spite of these errors, measurements have been found indispensable in comparing and identifying the 10 chromosomes of the monoploid set.

The measurements from the drawings of the chromosomes of a plant of an F_1 hybrid between Florida teosinte and maize are given in table 1. The same measurements were taken for each of the several plants of each teosinte strain used in determining the mean lengths and positions of other morphological characteristics of the chromosomes of the various strains of teosinte and teosinte-maize hybrids.

The lengths of the chromosomes (in centimeters), given in table 1, are derived from drawings magnified 3,000 times. The values given for the positions of the fiber-attachment region and for the positions of the internal knobs on the chromosomes represent the shortest distance between the attachment region or the knobs and the end of the chromosome, expressed as percentages of the total length of the chromosome. Thus, the total length of the first teosinte chromosome is 30.50 cm, and the spindle-fiber attachment region is 42.6 percent of the length from the nearest end. Consequently, the short arm of this chromosome is 42.6 percent and a long arm 57.4 percent of the total length of the chromosome. The terminal knob on the short arm is indicated by the letter T.

TABLE 1.—*Length and position of morphological characters of the chromosomes of Florida teosinte-maize F_1 hybrids*

Chromosome	Chromosome characteristics of—							
	Teosinte homologue				Maize homologue			
	Length	Position of fiber attachment	Knob position [1]		Length	Position of fiber attachment	Knob position	
			Short arm	Long arm			Short arm	Long arm
	Centimeters [2]	*Percent* [3]			*Centimeters* [2]	*Percent* [3]		*Percent* [3]
I	30.50	42.6	T	.....	30.50	42.6	.....	22.4
	22.00	45.4	T	.....	22.00	45.4	.....	22.7
	23.25	45.1	T	.....	23.25	45.1	.....	20.4
	26.50	42.4	T	.....	26.50	42.4	.....	22.6
	29.00	46.6	T	.....	29.00	46.6	.....	24.1
Average	26.25	44.4	.....	.....	26.25	44.4	.....	22.4
II	22.00	41.6	T	T	21.00	44.2	.....	22.6
	24.50	43.0	T	T	23.50	45.2	.....	19.4
	23.75	47.4	T	T	23.75	47.4	.....	23.1
Average	23.42	44.0	.....	.....	22.75	45.6	.....	21.6
III	21.50	36.7	T	.....	21.50	30.7	.....	26.6
	17.50	32.8	T	.....	17.50	32.8	.....	28.6
	24.50	34.1	T	.....	24.50	34.1	.....	33.3
	20.00	31.4	T	.....	20.00	31.4	.....	36.2
	28.75	29.5	T	.....	28.75	29.5	.....	31.2
	18.50	33.8	T	.....	18.50	33.8	.....	28.4
Average	21.79	33.0	.....	.....	21.79	33.0	.....	30.7
IV	24.00	42.8	T	T	23.00	44.7	.....	18.5
	16.75	38.8	T	T	16.75	38.8	.....	16.4
	19.50	33.3	T	T	18.50	35.1	.....	19.0
	19.00	44.7	T	T	19.00	44.7	.....	21.0
	21.75	40.9	T	T	20.75	39.0	.....	19.0
	22.75	39.9	T	T	22.75	39.9	.....	24.2
Average	20.63	40.0	.....	.....	20.13	40.3	.....	19.6
V	19.50	43.2	T	T	18.50	46.1	.....	21.6
	18.50	43.2	T	T	17.00	46.0	.....	19.1
Average	19.00	43.2	.....	.....	17.75	46.0	.....	20.3
VI	17.00	25.0	N	T	16.25	26.1	N	18.5, 26.2
	17.50	21.4	N	T	17.50	21.4	N	22.8
	16.00	21.9	N	T	16.00	21.9	N	25.0, 34.2
Average	16.83	22.7	.....	.....	16.58	23.1	.....	22.1, 30.2
VII	17.50	25.7	.....	T	17.50	25.7	.....	22.8
	13.00	23.1	.....	T	13.00	23.1	.....	32.7
	14.25	28.1	.....	T	14.25	28.1	.....	29.8
	18.75	28.0	.....	T	18.75	28.0	.....	29.3
	15.00	23.4	.....	T	15.00	23.4	.....	36.6
	13.50	25.9	.....	T	13.50	25.9	.....	30.4
Average	15.33	25.7	.....	.....	15.33	25.7	.....	30.2
VIII	13.75	21.5	.....	T	12.75	22.7	.....	11.4, 18.9
	14.25	21.5	.....	T	14.25	21.5	.....	14.0, 22.8
	20.75	25.3	.....	T	20.75	25.3	.....	15.0, 23.0
	15.50	20.9	.....	T	14.00	23.2	.....	17.7, 25.0
	17.25	20.3	.....	T	17.25	20.3	.....	18.7, 27.5
	17.75	18.6	.....	T	17.75	18.6	.....	18.3, 28.2
Average	16.54	21.3	.....	.....	16.13	21.9	.....	15.8, 24.2
IX	14.50	25.8	.....	T	12.75	29.4	.....	.....
	16.50	30.3	.....	T	14.25	35.0	.....	.....
	11.50	30.4	.....	T	10.25	34.1	.....	.....
	16.50	31.8	.....	T	14.25	30.0	.....	.....
Average	14.75	29.5	.....	.....	12.87	32.1	.....	.....
X	11.50	21.7	.....	T	10.00	25.0	.....	.....
	10.50	25.0	.....	T	10.00	25.0	.....	.....
	11.25	22.2	.....	T	11.25	22.2	.....	.....
	11.75	23.5	.....	T	11.75	25.5	.....	.....
	15.50	25.8	.....	T	15.50	25.8	.....	.....
	12.75	21.6	.....	T	11.75	23.4	.....	.....
	13.50	27.9	.....	T	13.50	27.9	.....	.....
	11.50	26.1	.....	T	11.50	26.1	.....	.....
Average	12.28	24.5	.....	.....	11.91	25.1	.....	.....

[1] T=terminal; N=at nucleolus.

[2] From drawings magnified × 3,000.

[3] Distance from fiber attachment or knob to nearer end of chromosome, expressed as percentage of total length of chromosome.

The diagrammatic representations of the chromosomes of the various strains of teosinte are based on averages of the measurements of all drawings of each individual chromosome. Usually only enough drawings were made of chromosomes from an individual plant to insure a representative picture. The diagram of a particular chromosome shows its length and the position of its fiber attachment and knobs.

FLORIDA TEOSINTE

To procure the best cytological material possible plants of Florida teosinte were grown in several localities. Plants flowering in the cold fall days at Torrey Pines, Calif., gave very few satisfactory preparations; the chromosomes were usually too compactly intertwined to be traced throughout their whole length, and the knobs were fused to such an extent that their size was indeterminable. Much better preparations were obtained from material grown in the more favorable environment at Coconut Grove, Fla.

Midprophases of pollen mother cells of Florida teosinte showed that prominent terminal knobs are characteristic of many of its 10 chromosomes. Considerable variation in the number and size of these knobs was found in sister plants, indicating that even after years of rather close breeding in Florida variations in the morphological characters of the chromosomes have persisted. These variations necessitated the inclusion of two types for chromosomes III, IV, VII, and VIII in the diagram of chromosomes (fig. 1).

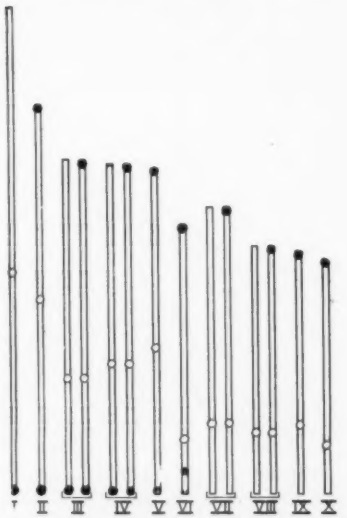


FIGURE 1.—Diagram of the 10 chromosomes of Florida teosinte, showing knobs (black) and fiber attachments (circles). Chromosomes III, IV, VII, and VIII are each represented by two types. × 1,000.

The arrangement of the chromosomes shown in figure 1 is based on a study of the maize and Florida teosinte homologues in two F_1 hybrid plants in which the pairing was usually regular. In many of the sister F_1 plants it was found that parts of chromosomes were unpaired and that the association of nonhomologous ends of chromosomes was frequent.

A diagram of the 10 Florida teosinte chromosomes and their 10 maize homologues, based on drawings of one of the more normal-behaving F_1 hybrids, is shown in figure 2, with the paired homologues drawn separately, side by side, the teosinte one always at the left. The relative lengths of the Florida teosinte chromosomes in this plant differ slightly from the average lengths shown in figure 1.

The terminal position of all knobs on the chromosomes of Florida teosinte, except the one adjacent to the point at which chromosome VI is attached to the nucleolus, is in marked contrast to the internal

position of the knobs on the associated maize chromosomes in this F_1 hybrid.

Differences in length found in the associated threads of this Florida teosinte-maize F_1 hybrid are shown in table 1. The table indicates that the teosinte and maize homologues are the same length only in chromosomes I, III, and VII. In some cases no difference was detected in the length of the two homologues, whereas a difference in length could be detected in the same chromosome in a sister cell. Such slight differences were usually not apparent in the more contracted chromosomes, and only in chromosomes V and IX were there consistent differences in the lengths of the associated homologues.

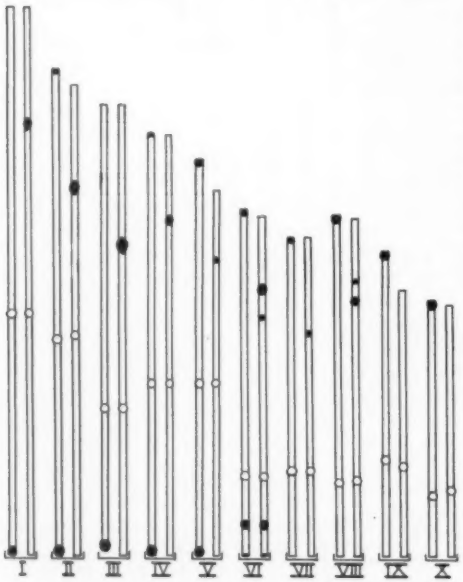
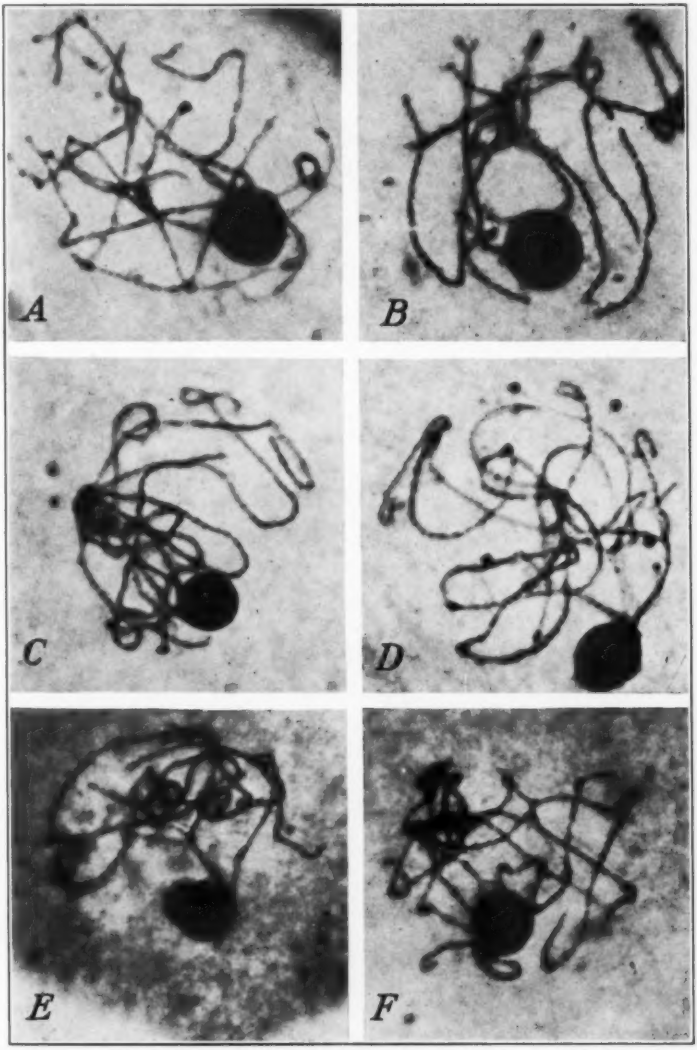


FIGURE 2.—Diagram of the 10 chromosomes of Florida teosinte × maize F_1 hybrid, showing knobs (black) and fiber attachments (circles). In each pair the teosinte homologue is on the left; the maize homologue, on the right. × 1,000.

A normal association of the teosinte and the maize chromosomes is shown in figure 3 and in the photomicrograph, plate 1, *A*, both made from the same cell. The maize parent of this hybrid had internal knobs on all but the two shortest chromosomes; this character is an aid in the identification of the paired homologues.

In the cell shown in figure 3, chromosome I is exceptional in that the short arm of the Florida teosinte homologue extends beyond the end of the associated maize chromosome. Chromosome III of this drawing shows the short arm of the maize homologue extending slightly beyond the terminal knob on the short arm of the Florida teosinte homologue. This difference in length of the short arm of chromosome III, although usually apparent, was too insignificant to affect the measurements given for this chromosome in table 1. Sometimes the end of the maize chromosome protruded only sufficiently to give the terminal knob on the teosinte chromosome an elongated appearance, while in other cells a short piece of the maize chromosome protruded beyond the teosinte knob. This short piece frequently has its terminal chromomere enlarged to form a small knob, as in figure 3, suggesting the transfer of some of the knob material from the teosinte chromosome to the nearby end of the maize chromosome.

Chromosomes V and IX of this hybrid show, more than the other chromosomes, an apparent lack of perfect homology of the associated



Midprophase stage of the first reduction division of pollen mother cells of annual teosinte strains. The cells are also shown in the following figures: *A*, In figure 3; *B*, in figure 6; *C*, in figure 7; *D*, in figure 10; *E*, in figure 15; *F*, in figure 18. Photomicrographs. All × 1,000.

teosinte and maize chromosomes. Chromosome V frequently is not closely associated throughout its whole length; sometimes this lack of association is along an internal region and at other times only at the ends. Chromosome IX invariably has the long arm of the teosinte chromosome considerably longer than the corresponding arm of the maize homologue, a difference in length that is sufficient to make the mean position given in table 2 for the fiber attachment of Florida teosinte lower than that given for maize.

The mean length of each chromosome and the mean position of the fiber attachments for the various teosintes and for the maize homologue in the Florida teosinte-maize hybrid shown in table 1 are given in table 2. These means are fixed only approximately as shown by the errors, and with more study might have been fixed more definitely

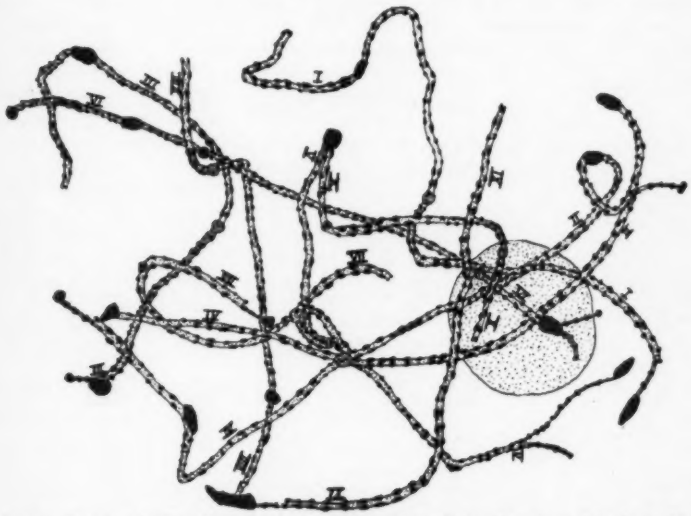


FIGURE 3.—Drawing of the 10 chromosomes of a pollen mother cell at midprophase of the first reduction division from a Florida teosinte × maize F_1 hybrid. × 2,000.

especially for the more readily identified chromosomes. The means were computed from data similar to those of table 1, except that the measurements were taken from several plants of each group instead of from a single plant.

Only a sufficient number of measurements of an individual chromosome were made to give an average value that would be useful in making a comparison of the 10 chromosomes that make the monoploid sets of the various teosinte strains. The most satisfactory measurements obtained for identifying and comparing chromosomes were from cells in which the length and position of the morphological characters of all 10 chromosomes could be determined. But such cells are hard to find, and the writer has resorted to the use of additional, more fragmentary data obtained from cells showing only a few of the chromosomes clearly. The data from all drawings of a chromosome have been combined for the determination of its mean length and the mean position of its other morphological characters.

TABLE 2.—*Length and position of fiber attachment of chromosomes of annual teosintes and maize*

Variety	Chromosome I		Chromosome II		Chromosome III	
	Length	Position of fiber attachment	Length	Position of fiber attachment	Length	Position of fiber attachment
	Centimeters [1]	*Percent* [2]	*Centimeters* [1]	*Percent* [2]	*Centimeters* [1]	*Percent* [2]
Teosinte:						
Florida	24.0±1.1	45.6±1.3	21.5±1.0	45.3±1.2	16.5±1.0	34.4±1.1
Progreso	21.8±1.1	40.2±1.3	21.4±1.2	45.6±1.4	18.9±2.3	34.4±2.5
Moyuta	22.8±1.2	44.6±1.5	18.2±1.1	43.5±1.3	17.4±1.2	35.6±1.4
Nojoyá	24.0±1.4	41.7±1.7	18.2±1.1	42.4±1.3	16.2±1.9	34.9±2.3
San Antonio	22.4± .6	45.1± .8	19.1± .8	40.0± .9	17.3± .9	35.6±1.0
Durango	19.8± .8	41.6±1.1	14.5± .6	46.4± .7	15.3± .7	33.3± .8
Chapingo	18.2± .8	45.0± .9	15.6± .6	43.9± .7	14.7± .8	31.0± .8
Maize	26.2±1.1	44.4±1.3	22.8±1.4	45.6±1.7	21.8±1.4	33.0±1.5

Variety	Chromosome IV		Chromosome V		Chromosome VI	
	Length	Position of fiber attachment	Length	Position of fiber attachment	Length	Position of fiber attachment
	Centimeters [1]	*Percent* [2]	*Centimeters* [1]	*Percent* [2]	*Centimeters* [1]	*Percent* [2]
Teosinte:						
Florida	16.5±1.2	38.8±1.4	16.5±1.2	44.8±1.6	13.7±0.6	20.9±1.0
Progreso	19.2±1.2	37.2±1.4	17.2±1.2	47.2±1.6	11.6± .8	22.4±1.2
Moyuta	17.2± .9	37.0±1.0	15.8±1.0	45.8±1.4	11.7± .8	22.2±1.3
Nojoyá	16.6±1.4	38.7±1.6	16.2±1.2	48.5±1.6	11.8±1.0	23.8±1.4
San Antonio	17.3± .8	40.2± .9	17.1± .7	44.4±1.0	12.9± .6	24.4± .9
Durango	14.2± .6	38.6± .7	15.0± .5	44.2± .7	11.3± .4	25.1± .6
Chapingo	14.7± .6	40.2± .7	14.3± .6	46.2± .8	10.9± .4	22.7± .6
Maize	20.1±1.0	40.3±1.1	17.8±1.8	46.0±2.3	16.6±1.1	23.1±1.6

Variety	Chromosome VII		Chromosome VIII		Chromosome IX	
	Length	Position of fiber attachment	Length	Position of fiber attachment	Length	Position of fiber attachment
	Centimeters [1]	*Percent* [2]	*Centimeters* [1]	*Percent* [2]	*Centimeters* [1]	*Percent* [2]
Teosinte:						
Florida	14.4±0.7	26.4±1.0	12.4±0.8	24.6±0.7	12.1±0.5	29.5±0.6
Progreso	14.4± .8	25.5±1.2	14.5±1.1	24.0±1.0	13.5± .7	25.8±1.0
Moyuta	12.5± .7	28.3±1.0	12.8±1.0	22.6± .9	12.6± .7	29.0±1.0
Nojoyá	12.2±1.3	30.6±2.0	12.6±1.1	26.4±1.7	11.7±1.2	34.8±1.6
San Antonio	13.9± .7	29.6± .6	14.7± .5	27.9± .8	12.2± .5	35.4± .7
Durango	10.3± .4	26.7± .5	10.8± .4	24.7± .4	9.4± .3	34.3± .4
Chapingo	10.6± .5	30.7± .8	11.6± .5	22.1± .5	10.7± .4	36.1± .5
Maize	15.3± .8	25.7±1.2	16.1±1.0	21.9±1.0	12.8±1.0	32.0±1.3

Variety	Chromosome X		Chromosome X-a	
	Length	Position of fiber attachment	Length	Position of fiber attachment
	Centimeters [1]	*Percent* [2]	*Centimeters* [1]	*Percent* [2]
Teosinte:				
Florida	11.7±0.5	22.5±0.7		
Progreso	11.2± .9	21.8±1.1		
Moyuta	10.2± .7	20.4± .9		
Nojoyá	10.5±1.0	27.5±1.4		
San Antonio	10.4± .5	25.8± .7		
Durango	8.6± .3	26.1± .4		
Chapingo	9.1± .4	27.6± .6	12.8±0.8	20.1±1.1
Maize	11.9± .7	25.1±1.0		

[1] Length from drawings magnified × 3,000.
[2] Distance from fiber attachment to nearer end of chromosome, expressed as percentage of total length of chromosome.

The position of the fiber attachments of Florida teosinte chromosomes as given in table 1 are in most cases sufficiently characteristic to place the chromosomes in the same order as that assigned to the 10 maize chromosomes.

It was frequently observed that unassociated ends of chromosomes in Florida teosinte-maize hybrids are double, while the associated parts of the same chromosome do not show any indications of a four-thread condition. The division of the unassociated ends of a chromosome in advance of the adjacent associated portion is illustrated in figure 3 and indicates that the division of a chromosome is retarded by its association with its homologue.

GUATEMALAN TEOSINTES

PROGRESO

For cytological material, plants of Progreso teosinte were grown from the first seed sent by Popenoe from southern Guatemala after the recent rediscovery of wild teosinte in that region. The chromosomes of Progreso and of Florida teosinte were very similar. A diagram of the 10 chromosomes of the monoploid set of Progreso teosinte is given in figure 4. The diagram shows two types of chromosome VIII, one with a large terminal knob and the other with a terminal knob the size of a large chromomere, a difference in size similar to that pictured for chromosome IV of Florida teosinte.

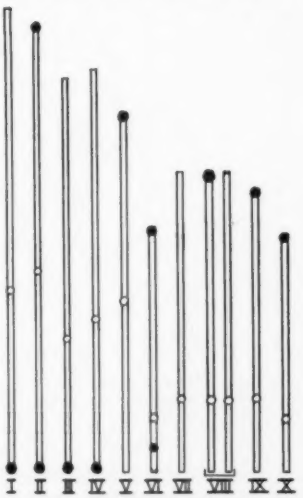


FIGURE 4.—Diagram of the 10 chromosomes of Guatemalan teosinte from Progreso, showing knobs (black) and fiber attachments (circles.) Chromosome VIII is represented by two types. × 1,000.

MOYUTA

In Moyuta, the second strain of teosinte from southern Guatemala, the arms of many chromosomes are terminated by knobs, and the 10 chromosomes are very similar to those of strains of teosinte mentioned above. The similarity of the morphological characters of the chromosomes of the two southern Guatemalan teosintes and of Florida teosinte indicates that all three strains had a common ancestor. The variability in the number and size of the terminal knobs on the chromosomes of plants within any of these three strains is only slightly less than that found in comparing plants from the different strains.

The characteristics of individual chromosomes of Moyuta teosinte at midprophase stage are shown in figures 5, 6, and 7, and in plate 1, *B* and *C*.

It is not unusual to find in teosintes distinct differences in the size of the knobs that terminate the two associated chromosomes and to find fusion of knobs or fiber attachments of nonhomologous chromosomes, such as are shown in figure 6. It is, however, unusual to find as many as 11 terminal knobs fused into one common mass of knob material, as shown in figure 7.

The general similarity of the chromosomes of the teosintes from Florida, Progreso, and Moyuta and their origin from the same general area in southern Guatemala have led to the grouping of these three teosintes together as southern Guatemalan strains.

NOJOYÁ

The few collections of cytological material of Nojoyá, a northern Guatemalan strain of teosinte, made by Popenoe and Kempton were not sufficient to determine the size of all terminal knobs. Consequently, in figure 8 certain knobs are shown as heavy circles when the fact that they were present was certain but their size was undetermined.

FIGURE 5.—Diagram of the 10 chromosomes of Guatemalan teosinte from Moyuta, showing knobs (black) and fiber attachments (circles). Chromosomes IV, VII, and VIII are each represented by two types. × 1,000.

This teosinte has fewer arms without terminal knobs, and these knobs are frequently no larger than large chromomeres. That these small knobs are true knobs is shown by their occasional fusion with each other and with larger knobs.

Nojoyá teosinte has knobs terminating the short arms of chromosomes VII, VIII, and IX, knobs that were not found on any of the plants from southern Guatemala. Some strains of maize have knobs terminating the short arms of chromosomes VII and IX. Consequently the shorter chromosomes of Nojoyá teosinte are more like the corresponding chromosomes of maize than are those of the southern Guatemalan strains.

SAN ANTONIO

Because of limited material and the frequent fusion of knobs it was not possible to determine the size of all terminal knobs in each plant of San Antonio, the second strain of teosinte from northern Guatemala. This failure to make a definite determination of knob size has made it necessary to increase the number of types of most chromosomes shown in figure 9.

The terminal knobs of San Antonio teosinte vary in size and number from plant to plant, but, as in all teosintes, they are approximately constant in size within a plant. The midprophase of a pollen mother cell is shown in figure 10 and plate 1, *D*. The drawing and the photomicrograph show a few peculiar features such as differences in the sizes of terminal knobs, the fusion of some of the knobs, the fusion of fiber-attachment regions, and two small internal knobs, one on the short arm of chromosome IV and the other on the long arm of chromosome VI. The internal knobs shown are not prominent and, especially on chromosome IV, are only a little larger than large chromomeres.

The presence of internal knobs on the chromosomes of two plants of San Antonio teosinte may be explained by the possible admixture

of maize in their ancestry, an explanation that is supported by a sample of seed recently received from this locality that included a few spikes clearly showing evidence of hybridization with maize.

The positions of the fiber attachments of the longer chromosomes of both northern Guatemalan strains, as shown in table 2, are approximately the same as those given for the southern Guatemalan strains and for maize. The fiber attachment of chromosome IX, however, is

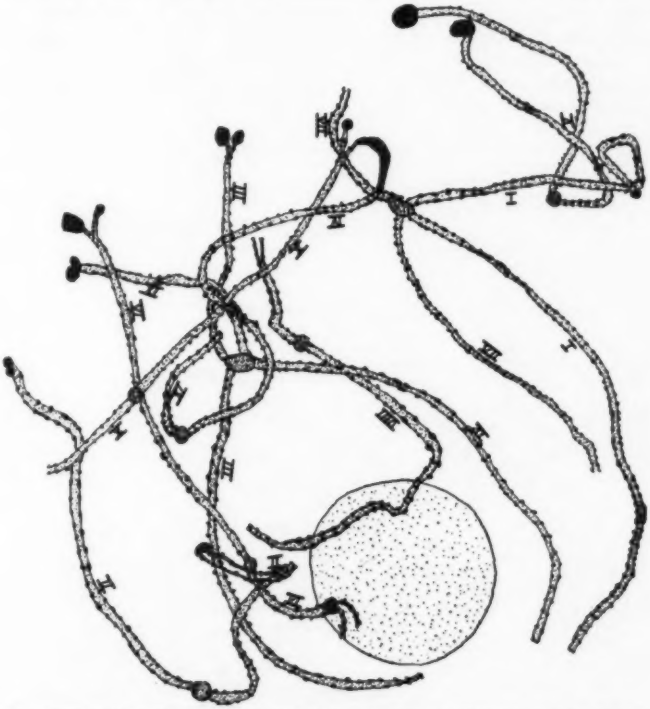


FIGURE 6.—Drawing of the 10 chromosomes of a pollen mother cell at midprophase of the first reduction division of Guatemalan teosinte from Moyuta. × 2,000.

further from the end of the chromosome than it is in maize or in southern Guatemalan strains.

San Antonio and Nojoyá teosintes may be considered as approximately identical and represent strains that will be designated as northern Guatemalan teosintes. However, San Antonio teosinte, with internal knobs in two plants and with both strains characterized by knobs terminating the short arm of several of the shorter chromosomes, is distinguishable from the southern Guatemalan strains and has characters that suggest either an admixture of maize or a closer relationship to maize or both.

MEXICAN TEOSINTES

PERENNIS

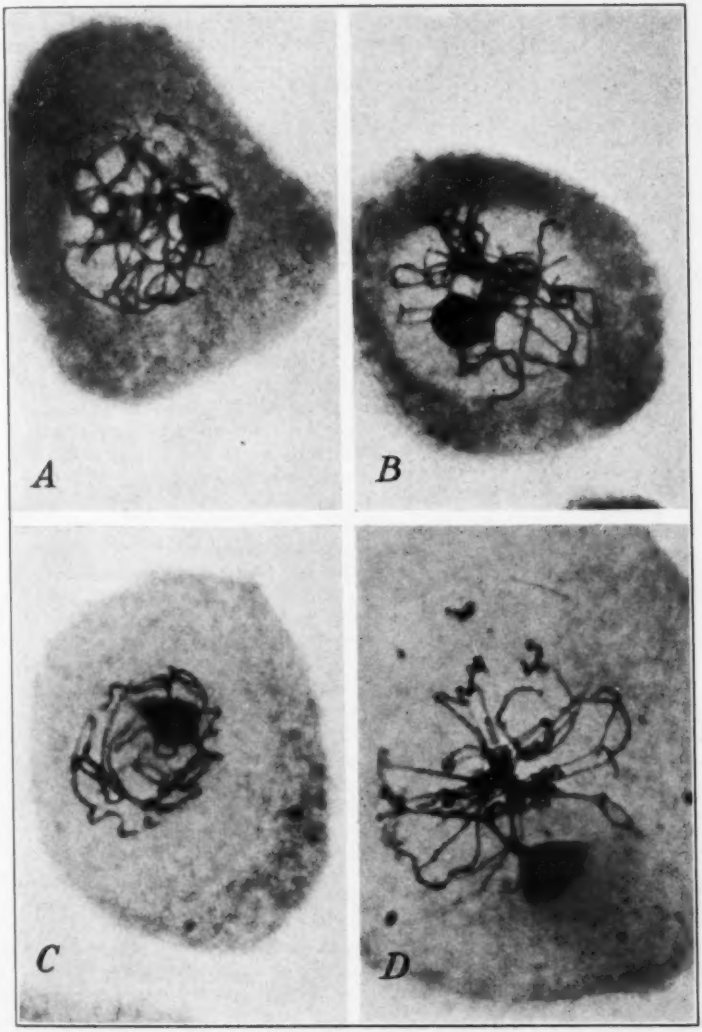
The much-intertwined thread stages of the pollen mother cells of perennial teosinte (*Euchlaena perennis*) and the presence of twice as many chromosomes as in the annual form made it difficult to find isolated chromosomes or groups of chromosomes that could be identified. A diagram of the monoploid set of 10 chromosomes which are replicated in this tetraploid teosinte is shown in figure 11. The length of the chromosomes and the position of the fiber attachments of this diagram are based on relatively few drawings.

Chromosome identification was made more difficult by the absence of distinguishing knobs, except on chromosome VI adjacent to the

FIGURE 7.—Drawing of the 10 chromosomes of a pollen mother cell at midprophase of the first reduction division of Guatemalan teosinte from Moyuta, showing the fusion of 11 knobs into one large knob mass. × 2,000.

region attached to the nucleolus. The knobs, if they may be called knobs, were nothing more than prominent chromomeres. In no case were these enlargements united with those on other chromosomes, as it might be expected they would be occasionally if they were true knobs.

In this study of the chromosomes of perennial teosinte, material was available also of a perennial teosinte contributed by Randolph, according to the Press Service (*9*), that arose as a mutation from annual teosinte. Midprophase stages of the chromosomes of this plant failed to show any morphological characters that could distinguish it from the chromosomes found in the plants of perennial



Midprophase stage of the first reduction division of tetraploid pollen mother cells from perennial strains of teosinte: *A*, Originated by Randolph, and *B*, introduced from Mexico in 1921; diploid *C* and tetraploid *D* pollen mother cells of Mexican teosinte from Durango. Photomicrographs. All × 1,000.

teosinte introduced from Mexico by Collins and Kempton as reported by Collins (*3*). A photomicrograph of a pollen mother cell at midprophase of the first reduction division from each of these types of perennial teosinte is shown in plate 2, *A* and *B*. The chromosome threads in both perennial forms are without prominent terminal knobs.

DURANGO

The chromosomes of Durango teosinte were found to be more uniform than those of any other form.

The seed used in this study has been grown for years in this country, and the lack of variability in the morphological characteristics of its chromosomes, found in a plant-to-plant study, may be accounted for by inbreeding through occasional self-fertilization.

The morphological characters of the chromosomes of this strain of teosinte are decidedly more like maize than any of the teosintes previously described. The dearth of terminal knobs, which are so prevalent in Guatemalan teosintes, and the presence of internal knobs on many of its 10 chromosomes are shown in figures 12 and 13. Figure 13 also shows a B-type chromosome that has doubled back on itself. In some of the plants of this strain of teosinte there were as many as eight B-type chromosomes, frequently in paired association, but in many cases several were united in a complicated tangle.

Durango teosinte is generally considered to show maizelike characteristics that suggest repeated hybridization with maize. The general similarity of the chromosomes of Durango teosinte and maize are shown in figure 14, which is a diagram of the homologous chromosomes from an F_1 maize-Durango teosinte hybrid. The length of the associated chromosomes of the F_1 hybrid seemed to be the same, and the internal knobs present on the teosinte chromosomes were in approximately the same position as those found on the maize chromosomes.

FIGURE 8.—Diagram of the 10 chromosomes of Guatemalan teosinte from Nojoyá, showing knobs (black) and fiber attachments (circles). Knobs the size of which was not determined are represented by heavy circles. × 1,000.

The maize parent of this hybrid lacked a few of the knobs found in the teosinte chromosomes, but another maize might have been chosen as a parent in which the chromosomes would have agreed more closely in their morphological characters with those found in this Durango teosinte.

The usual close association of all chromosomes of maize and Durango teosinte are shown in figure 15 and plate 1, *E*. Rarely cases were found in which the association of two or more homologues was incomplete, but in general the pairing in this hybrid was as normal as is usually found in maize.

One Durango teosinte plant used in this study was unusual in having many pollen mother cells with double the normal number of

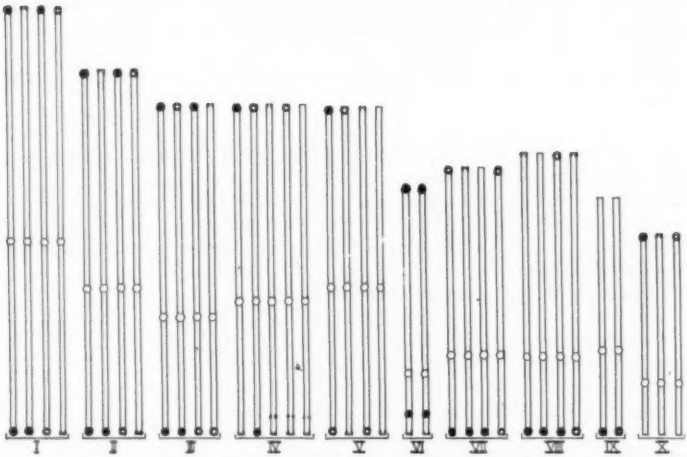


FIGURE 9.—Diagram of the 10 chromosomes of Guatemalan teosinte from San Antonio Huixta, showing knobs and fiber attachments. From two to five types are shown for each chromosome. In those types in which the knobs are shown as heavy circles the knob size was not determined. × 1,000.

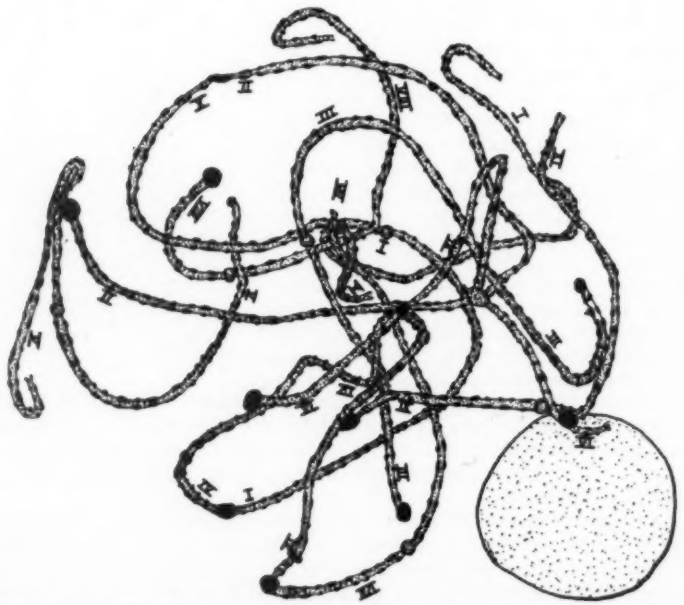


FIGURE 10.—Drawing of the 10 chromosomes of a pollen mother cell at midprophase of the first reduction division of Guatemalan teosinte from San Antonio. × 2,000.

chromosomes. One preparation from this plant showed almost 50 percent of the cells tetraploid. These tetraploid cells were carefully

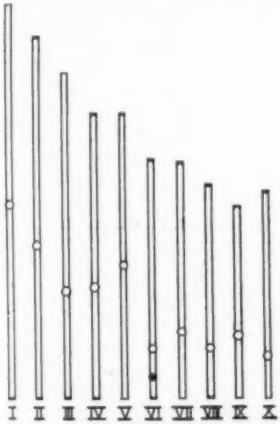


FIGURE 11.—Diagram of 10 of the 20 chromosomes of *Euchlaena perennis*, showing diminutive terminal knobs (black) and fiber attachments (circles). × 1,000.

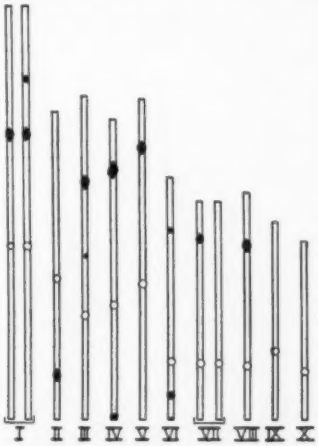


FIGURE 12.—Diagram of the 10 chromosomes of Mexican teosinte from Durango, showing knobs (black) and fiber attachments (circles). × 1,000.

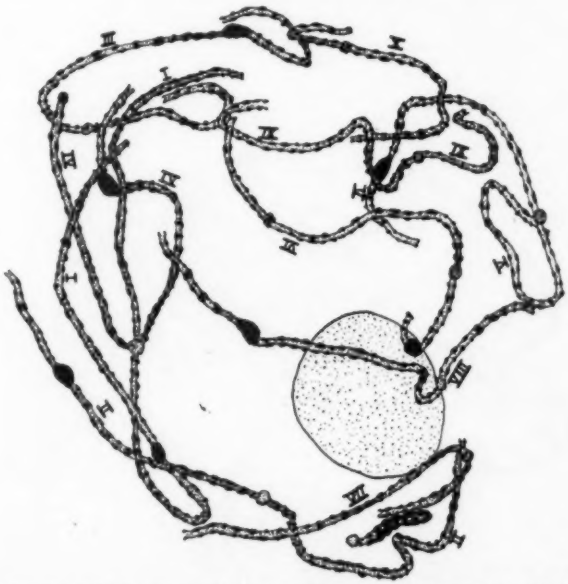


FIGURE 13.—Drawing of the 10 chromosomes of a pollen mother cell at midprophase of the first reduction division of Mexican teosinte from Durango. × 2,000.

studied, and plate 2, *C* and *D*, shows the midprophase stage of a diploid and a tetraploid cell, respectively. *A* shows a tetraploid cell

from Randolph's perennial teosinte obtained from seed of annual teosinte, and *B* shows a tetraploid cell of perennial teosinte introduced from Mexico in 1921. The perennial teosinte of Randolph, the original perennial teosinte from Mexico, and the tetraploid cells in the Durango teosinte plant all have their bivalent chromosomes in duplicate, and the last-named plant has a bivalent B-type chromosome instead of the single B-type chromosome found in the sister diploid cells of this Durango plant. These photomicrographs show the knobs marking the Durango teosinte chromosomes in both the diploid and tetraploid cells that occurred in the same plant, while the chromosomes of the two types of perennial teosinte are practically

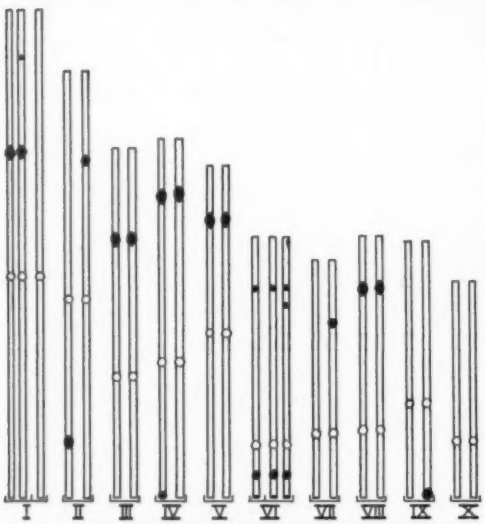


FIGURE 14.—Diagram of the 10 chromosomes of maize × Durango teosinte F_1 hybrid, showing knobs (black) and fiber attachments (circles). In each group the teosinte homologue is shown on the left, the maize homologue on the right. Chromosome I has two types of the teosinte chromosome and chromosome VI has two types of the maize chromosome. × 1,000.

FIGURE 15.—Drawing of the 10 chromosomes of a pollen mother cell at midprophase of the first reduction division of maize × Durango teosinte F_1 hybrid. × 2,000.

identical and are marked only by the internal knobs on chromosome VI adjacent to the point where they are attached to the nucleolus.

CHAPINGO

Chapingo teosinte, though very distinct from Durango, suggests in many of its morphological characters an admixture of maize.

The chromosomes from plants of each of three lots of seed received through Walter Douglas, who obtained them at the Government station at Chapingo, not far from Mexico City, varied morphologically; consequently figure 16 shows several types for most of the 10 chromosomes.

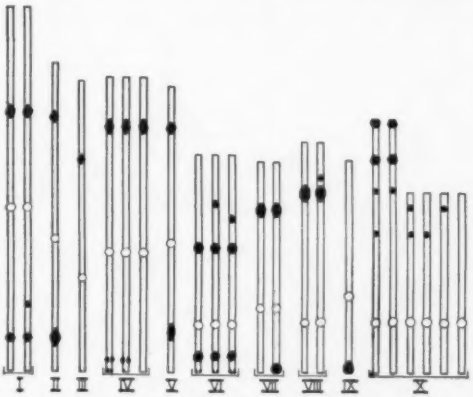


FIGURE 16.—Diagram of the 10 chromosomes of Mexican teosinte from Chapingo, showing knobs (black) and fiber attachments (circles). Chromosome X has both the long and short types represented, and several of the other chromosomes have more than one type. × 1,000.

The chromosomes of this group of teosintes (fig. 16) might be said to have all the knobs found on all the strains of maize put together and a few knobs that have never yet been reported in maize. Figure 17 shows the chromosomes of a strain of maize that has many of its chromosomes marked by knobs, which in number and distribution are very similar to the knobs shown in figure 16.

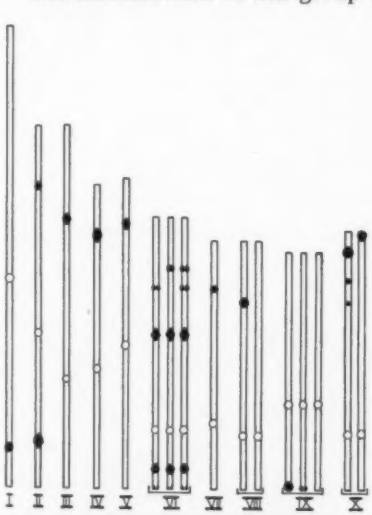


FIGURE 17.—Diagram of the 10 chromosomes of a strain of maize showing knobs (black) and fiber attachments (circles). Chromosomes VI, VIII, IX, and X are represented by more than one type and chromosome X is abnormally long. × 1,000.

The chromosomes of Chapingo teosinte, like those of Durango, have very few morphological characteristics that would link this strain with any of the Guatemalan strains and many that suggest a recent admixture of maize.

The much-knobbed character of the 10 chromosomes of Chapingo teosinte is shown in figure 18 and in plate 1, *F*. The tenth chromosome of the cell shown in these illustrations is unusual in having its long arm marked by two small knobs although the outer one is only on one of the associ-

ated threads. Still more unusual is a longer type of this shortest chromosome, found in each of four plants of this variable strain of teosinte.

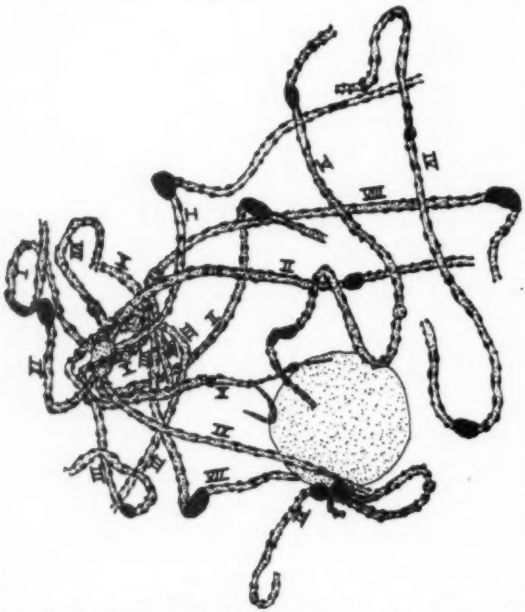


FIGURE 18.—Drawing of the 10 chromosomes of a pollen mother cell at midprophase of the first reduction division of Mexican teosinte from Chapingo. × 2,000.

It was surprising to find that the maize illustrated in figure 17 has two unusual types of chromosome X that are very similar to, though not identical with, the long types found in Chapingo teosinte.

The origin of the much-knobbed, longer types of chromosome X might be the result of the addition of a fragment marked by both a terminal and an internal knob, or the picture might be reversed by considering that the normal short types have resulted from the loss of a terminal portion of the long arm of the long type.

TRIPSACUM

A preliminary study of the morphological characters of the chromosomes of *Tripsacum floridanum* Porter and the diploid form of *T. dactyloides* (L.) L. has been made. Figure 19 shows the 18 chromosomes of the former species. The study has not reached the stage where a general comparison of the morphological markings of *Tripsacum* with teosinte and maize can be made. However, the presence of terminal knobs on all of the chromosomes suggests teosinte rather than maize. Chromosome XVI,

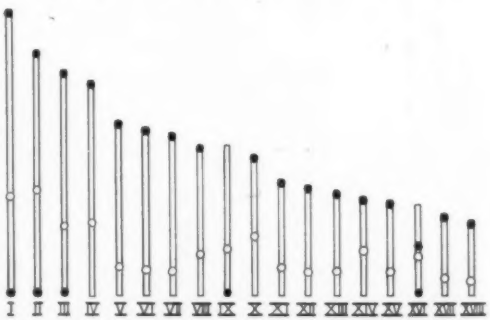


FIGURE 19.—Diagram of the 18 chromosomes of *Tripsacum floridanum*, showing knobs (black) and fiber attachments (circles). × 1,000.

attached to the nucleolus, is easily distinguishable in *Tripsacum* and differs from the similarly attached chromosome in both teosinte and maize in having its fiber attachment near the middle and in having a much longer piece extending beyond the point where it is attached to the nucleolus.

SORGHUM

The presence of knobs on the chromosomes of maize and its nearer relatives led to an examination of some of the more distantly related grasses. Fortunately, at the time these studies were being made, a sorghum introduced from Colombia and noted for the popping quality of its seeds was growing in the greenhouse at Washington and was in the proper condition to collect cytological material of the midprophase stage of the pollen mother cells.

These sorghum chromosomes show very distinctly the region of spindle-fiber attachment as a clear area with a deeply staining thread on either side, the staining quality of the thread weakening very appreciably a short distance on either side of the spindle-fiber attachment and the rest of the thread staining rather poorly. All 10 chromosomes of this sorghum are unmarked by knobs, and the longest chromosome was found to be attached to the nucleolus at a point near its middle and only a short distance from the spindle-fiber attachment (fig. 20). A second grain sorghum studied was found to be very similar to the strain described above. The absence of knobs on the chromosomes of this more distant relative of maize suggests the desirability of a study of some of the Andropogoneae, not so far removed from maize in their taxonomic characters as is sorghum.

FIGURE 20.—Diagram of the 10 chromosomes of a popping sorghum, showing fiber attachments (circles) and the region at which the longest chromosome is attached to the nucleolus (dotted circle). × 1,000.

KNOBS

Knobs, so far as the writer knows, are found only on the chromosomes of maize, *Euchlaena*, and *Tripsacum*, and have never been reported on the chromosomes of any other plant. Their presence on maize chromosomes at the midprophase stage of the first reduction division has proved very helpful in identifying the individual chromosomes that make up the chromosome complement of maize.

The discovery of knobs is so recent that as yet there has been no attempt to determine their composition or function. Therefore only a few characteristics of these dark-staining bodies are discussed herein.

KNOBS AS LARGE CHROMOMERES

It has been observed frequently that knobs on the chromosomes of one plant may be large, whereas in sister plants knobs in corresponding positions are little if any larger than prominent chromomeres.

This suggests that knobs are in fact large chromomeres. On chromosome VI of maize there has often been observed a chromomere near the middle of the long arm that is more prominent than its fellows. Recently the writer has found in several strains of maize and Chapingo teosinte a large knob in the position of this prominent chromomere.

Perhaps even more suggestive are the variations in size of the terminal knob of chromosome IX of maize and of several chromosomes of teosinte strains from Guatemala. In some plants, the terminal knob may be large and prominent; in other plants of the same strain this knob, while still conspicuous, is but little larger than a large chromomere; while in still other plants there is no noticeable knob and the terminal chromomere is not even prominent.

LENGTH OF CHROMOSOME INCREASED BY KNOBS

Many plants were studied in which one of the paired homologues was without a knob and the other had a prominent knob. That knobs are not superimposed on the thread but are enlargements and elongations of some region of the thread such as a chromomere is suggested by the pairing of homologues, one with and the other without a knob. It would appear that the chromosome is lengthened when a knob develops. The pairing of sections on either side of the knob-bearing region of such homologues leaves segments that include the knob region of unequal length and causes the chromosome to bend sharply or to leave an open loop or to push the knob to one side. Figure 21, *A*, consists of two drawings of chromosome III from maize, one bent sharply and the other with an open loop that overcomes the disparity in the lengths of the associated threads, while figure 21, *B*, shows a third method of adjustment so as to permit close association of homologous parts of the chromosomes.

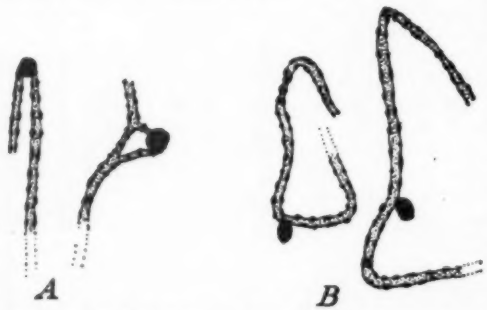


FIGURE 21.—Portions of chromosomes having a knob on only one homologue. Close association on either side of the knob is made possible (*A*) by bending and by the formation of a loop in the knob-bed homologue and (*B*) by the position of the knob off to one side, where it does not lengthen the chromosome appreciably. × 2,000.

CONSTANT POSITION OF KNOBS ON CHROMOSOME

It has been assumed that the position of a knob on a particular chromosome is constant in different cells of the same plant and in cells from closely related plants. The present study indicates that this constancy extends to such distantly related plants as maize and teosintes from Durango, Chapingo, and San Antonio.

There is, of course, variation in the measurements of knob position, but the variation in the position of a particular knob is the same whether the chromosomes are taken from cells of a single plant, from cells of sister plants, or from cells of the several kinds of teosinte and maize. Variation in the position of a particular knob seems largely

due to sectional variation in the elongation of a chromosome. If a hybrid is studied it is found that knobs coincide and are not separated by fractional pieces of the chromosome as would be the case if the knobs actually occupied slightly different positions.

The variation in position of the three knobs on the long arm of chromosome VI, designated as knobs 1, 2, and 3, has been subjected to statistical analysis. In general it was found that the total variation in position was distributed normally in the several subgroupings of the population of measurements; i. e. cells, plants, strains, and species. In the case of knob 1 the variation between species was on the border line of statistical significance. There also was some indication that knob 3 was somewhat more variable in location than the other two knobs. Since, however, knob location is expressed as a ratio of the

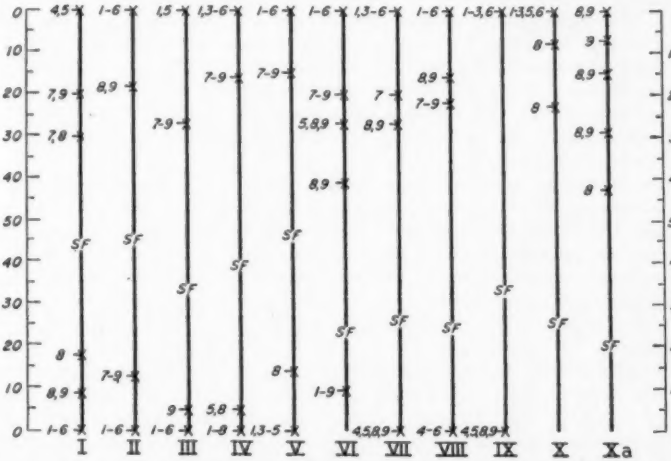


FIGURE 22.—Diagram of the 10 chromosomes of teosinte and maize, with the positions of knobs indicated by crosses (x) and those of fiber attachments by gaps (*SF*=spindle-fiber attachment). Chromosome X is shown in both its long type (Xa) and its short type (X). The numbers beside each knob position indicate the various strains, as follows: 1, Florida teosinte; 2, Progreso teosinte; 3, Moyuta teosinte; 4, Nojoyá teosinte; 5, San Antonio teosinte; 6, perennial teosinte; 7, Durango teosinte; 8, Chapingo teosinte; 9, maize. (For example, each of the strains from 1 to 6 has a knob terminating the short arm of chromosome 1.) The scales at the sides aid in measuring the distance knobs and fiber attachments are from the end of the chromosomes.

total length of a chromosome and the mean position of knob 3 is close to 0.5, whereas the other two knobs have mean positions close to 0.2, a correction for this difference in mean position must be made in the standard deviations.[3] When these corrections were made the apparent difference between knob 3 and the other two in variability of position largely disappeared. The generalized standard deviations of single observations for the positions of the three knobs are 0.03, 0.03, and 0.05, respectively.

Concluding, therefore, that knobs occur at certain definite positions on a chromosome, the writer gives diagrammatically in figure 22 the knob positions on the 10 chromosomes of the various teosintes included in this study and of several strains of maize. The knob positions for the various teosinte strains in this diagram are taken from the diagrams

[3] The method for making this correction is to multiply the observed σ by $0.5/pq$.

illustrating the position of the morphological characters on the chromosomes of these strains, and to these positions are added the various knob positions that have so far been found on several representative strains of maize.

Figure 22 is included only as an outline to be expanded as more material is critically examined. The usual preparation shows the major morphological characters of a chromosome, but the finer details are frequently overlooked except in the better preparations. Unquestionably there are many minor details yet to be mapped, and probably more major markers will be found.

GENETIC INERTNESS OF KNOBS

McClintock (*8*) called attention to the tendency for knobs to stick together. This tendency seems to vary greatly from cell to cell and from plant to plant. Whether stickiness of knobs is a plant characteristic or the result of some environmental conditions at the time cytological material is collected is uncertain. It has been found that chromosomes are more likely to be clumped in material collected in a cool environment, but this evidence does not eliminate the possibility that there may be individual plant differences with respect to the stickiness of the knobs.

The frequent stickiness of knobs and the behavior of B-type chromosomes when several are found associated at the midprophase suggest that knobs and B-type chromosomes have much in common. It is not unusual to find B-type chromosomes united at several points or for some distance along their length. McClintock (*8*) has published photographs and drawings of B-type chromosomes to illustrate the association of nonhomologous portions of chromosomes. The association of more than two threads of a B-type chromosome is explainable if the heavily staining portion of the B-type chromosome is composed largely of sticky knoblike material. This stickiness would possibly account for some of the nonhomologous association of the two ends of the same chromosome of McClintock's material.

Four drawings from a Durango teosinte plant carrying six B-type chromosomes are shown in figure 23. These drawings were selected because they illustrate adhesion of these chromosomes to each other. Figure 23, *A*, shows two bivalent B-type chromosomes united at the fiber-attachment region, and fused together again for about one-half their length, while the third bivalent is fused with one of these over a short section of the distal end. Figure 23, *B*, shows the three paired B-type chromosomes in which the fusion of the right-hand one has taken place without regard to the matching of homologous sections. Figure 23, *C*, pictures two unpaired B-type chromosomes doubled back on themselves, while the two paired B-type chromosomes are together in reverse order for a short portion of their central region. Figure 23, *D*, shows all three paired B-type chromosomes united in one region—the right-hand bivalent attached to the others only at the fiber-attachment region and at its distal end, while the other two are in close association for over half their length. These figures clearly indicate a general stickiness of these chromosomes over much of their length. The chromatic region of B-type chromosomes described by McClintock is the only region showing distinct chromomeres. This region is rarely seen in abnormal associations. The writer suggests that the abnormal associations prevalent in B-type chromosomes are

due to the presence of knoblike material which causes them to stick together rather than to a true association of homologous parts.

The presence of from one to many B-type chromosomes in a maize plant does not affect its morphological characters. If B-type chromosomes are largely made up of knoblike material, this would suggest that knobs are inert material. Koltzoff's (*5*) idea that the chromomeres making up the linelike markers on chromosomes of the salivary glands of *Drosophila* may be the inactive part of the chromosomes is in accord with the suggestion that knob material is inert genetically.

PERSISTENCE OF KNOBS ON TEOSINTE CHROMOSOMES AT PROPHASE OF SECOND REDUCTION DIVISION

Knobs on the chromosomes of maize are seen distinctly only in the early prophases of the first reduction divisions. In teosinte the terminal knobs are not so transitory and usually are present as distinctive markers terminating the chromosomes even as late as the prophase of the second reduction division. Figure 24 is a drawing of the chromosomes of a cell of a southern Guatemalan type of teosinte at this stage. Identification of the chromosomes at this phase is difficult without a previous knowledge of these chromosomes as seen in midprophases of the first division. Such pictures usually show several nucleoli instead of the one centralized nucleolus associated with chromosome VI. In a few of the better preparations the chromosomes show the spiral twisting of the chromonema, a detail that the writer has made no attempt to reproduce in his drawings. These figures are also clear illustrations of the interruption of the chromonema of the two arms of the chromosome by the fiber-attachment region. This phase, when showing a split chromosome with arms of very unequal length, led to the false impression that this chromosome is made up of two units of very unequal length. A case of nonhomologous threads is shown in figure 24 for chromosome VII; one long arm terminates in a knob, whereas the other is without a knob. Such a nonhomologous condition must be due either to an equational separation in the first division or to a cross-over.

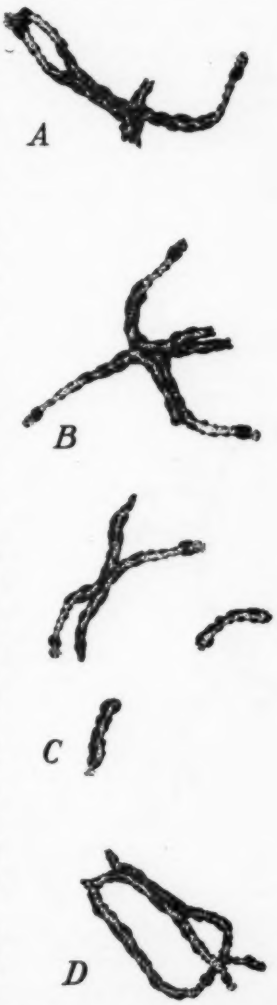


FIGURE 23.—Drawings showing the association or sticking together of six B-type chromosomes of Mexican teosinte from Durango: *A*, The sticking together, especially of the deeper staining homologous portions, of the bivalent chromosomes; *B*, the sticking together of both homologous and nonhomologous portions of the bivalent chromosomes; *C*, two univalent chromosomes folded back on themselves and two bivalent chromosomes with nonhomologous portions stuck together for a short distance; *D*, two bivalent chromosomes stuck together for one-half their length and a third attached to one of these in two regions. × 2,000.

DISCUSSION

Years of patient research will be required before all the morphological characteristics of each of the 10 chromosomes of maize can be described. McClintock (*7*, *8*) has described many distinguishing markers on the individual chromosomes that make up the normal set. The writer has been able to add a few more after a search of several strains of maize from the southwestern region of the United States. With these as a background for comparison, the present study of the morphological characters of the chromosomes of teosinte was started.

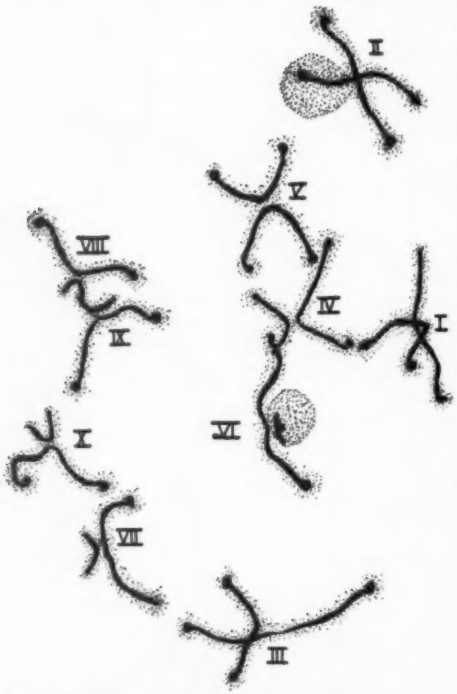


FIGURE 24.—Drawing of the 10 prophase chromosomes of the second reduction division of a pollen mother cell of southern Guatemalan teosinte, showing the persisting terminal knobs. × 2,000.

Beadle (*2*) has given a diagrammatic representation of the chromosomes of Florida teosinte. The arrangement of teosinte chromosomes would seem in better agreement with his diagram of the maize chromosomes if the third chromosome from the top were put as the fifth and the two bottom chromosomes changed places—an order suggested by the comparison of Florida teosinte with its maize homologues in an F_1 hybrid.

Florida teosinte, according to the historical records, was taken from southern Guatemala to France and later introduced into Florida. In comparing its chromosomes with those of two strains from southern Guatemala, a general similarity has been shown. Although no form is constant in its morphological characters, all show terminal knobs, and all have one internal knob on the short arm of chromosome VI adjacent to the nucleolus. The positions of the fiber attachments in the several chromosomes of these three teosintes agree as closely as could be expected from the method used in making these determinations, and the morphological characteristics of the chromosomes support the view of a common origin for this group of teosintes.

The chromosomes of teosintes from northern Guatemala show many of the markings found on the chromosomes of teosintes from southern Guatemala. Three points of difference that may be significant are: (1) The small terminal knobs on several chromosomes, a kind of knob

observed in the southern Guatemalan strains in only two instances; (2) the presence of terminal knobs on the short arm of three of the four shortest chromosomes, accompanied by a corresponding shift of the fiber attachments a little further from the end of the chromosome; and (3) the first indications of internal knobs; these internal knobs were not prominent and appeared only as enlarged chromomeres but were constant in their occurrence and position in two of the plants.

When the chromosome characteristics are compared, it seems clear that all the annual teosintes from Guatemala have much in common. The presence of knobs on the short arms of the shorter chromosomes of teosintes from northern Guatemala, however, distinguishes this group from the teosintes of southern Guatemala.

A comparison of the knobs on the chromosomes of Guatemalan teosintes with those on maize shows that the southern Guatemalan strains have only two knobs in positions that coincide with those known on maize chromosomes. One of these knobs terminates the short arm of chromosome I, a knob that is seen on this chromosome in only a few strains of maize. The second knob is the one on chromosome VI adjacent to the region where this chromosome is attached to the nucleolus, a knob that is universally present in this position in maize and all its near relatives.

The teosinte types from northern Guatemala have, in addition to the two knobs mentioned above, knobs terminating the short arm of chromosomes VII and IX. The latter knob is frequently found on the ninth chromosome of maize, and the former has been found on the seventh chromosome of several strains of maize.

Two plants from northern Guatemala gave the first indication of internal knobs in teosinte. These were on the long arm of chromosomes IV and VI in positions that coincide with the positions of knobs found on the chromosomes of maize. However, recent evidence that the teosintes of this area show some indications of contamination with maize makes it doubtful whether the internal knobs on the chromosomes of these two plants are of teosinte origin.

The chromosomes of maize have been arranged according to their length. Length alone is not always an entirely satisfactory method of identifying chromosomes because of the difficulty of having all chromosomes and all parts of chromosomes equally extended. The writer has found that chromosomes VII and VIII of maize vary in length and are so nearly the same length that if it were not for the slight differences in the position of the fiber attachment and the knobs that frequently mark them they would be indistinguishable. The mean lengths for the strains of teosinte studied and for maize are listed in table 2. It will be seen that chromosome VI of both the southern and the northern Guatemalan strains of teosinte is shorter than some of the chromosomes placed below it. However, the difference disappeared when this chromosome from Florida teosinte was studied in association with its maize homologue in an F_1 plant, and in one drawing of chromosome VI its length exceeded the maize chromosome, indicating that chromosomes have the ability to extend when necessary to bring about gene-to-gene pairing.

The length of the individual chromosomes of Florida teosinte has been compared with that of their maize homologues. This compari-

son has shown that all the chromosomes of Florida teosinte frequently are slightly longer than their maize homologues except chromosome III, the short arm of which is slightly shorter than the corresponding arm of the maize homologue.

Arnason (*1*) has used an entirely different method of showing the homology of Florida teosinte and maize chromosomes, and the writer agrees with his conclusions that chromosomes V and IX in an F_1 Florida teosinte-maize hybrid do not mate as well as do the other chromosomes. This difference in homology is shown in the writer's study by an appreciable difference in the length of these two chromosomes and by the fact that chromosome V has a frequent tendency toward a weak association with its maize homologue.

The tendency toward close association of maize and Florida teosinte chromosomes, however, seems to vary greatly from plant to plant in an F_1 progeny grown the past season. Many plants gave the impression that lack of pairing was the rule, while a few plants showed little or no incompatibility between maize and teosinte homologues.

The position of the fiber attachment divides each chromosome into two arms, the relative lengths of which in all Guatemalan teosintes are approximately those found for the corresponding maize chromosome, except in the shorter chromosomes such as chromosome IX. Even the slight differences in the positions of the fiber attachments are never so great that teosinte chromosomes do not fall naturally into the same order as the corresponding chromosomes of maize when the positions of the fiber attachments alone are taken into consideration.

The chromosomes of Guatemalan teosintes consequently differ most strikingly from the corresponding maize homologues in the position of knobs, and even the northern Guatemalan strains have only a few knobs in positions found on maize chromosomes.

Perennial teosinte is a tetraploid species in which the whole chromosome complement has been doubled and in which the terminal knobs are of minimum size. The length of the chromosomes and the position of the fiber attachments of this teosinte are approximately the same as those found in any strain of annual teosinte yet studied, but the absence of conspicuous terminal knobs separates it clearly from its diploid relatives.

Durango teosinte and the teosintes from the region around Mexico City stand apart from the Guatemalan teosintes just discussed. The morphological markings on their chromosomes are more nearly those characteristic of maize than of the chromosomes from the teosintes from Guatemala.

Durango teosinte has only one terminal knob on the short arm of chromosome IV that coincides with the knobs of southern and northern Guatemalan teosintes, while all the internal knobs, except possibly one on the long arm of chromosome I, are in approximately those positions occupied by knobs on the chromosomes of maize.

The knobs on the chromosomes of several forms of Chapingo teosinte received from Mexico City are in positions that coincide with those of the knobs found on maize chromosomes and show only a few indications of relationship to the northern Guatemalan teosintes and almost none to the southern Guatemalan strains.

The Chapingo strain of teosinte was striking because of the prevalence of internal knobs and an unusually long form of chromosome X found in a few plants. The unusual form of this shortest chromo-

some, in addition to being longer than usual, is marked by two prominent knobs, one at the end and the other near the end of the long arm.

Internal knobs on the chromosomes of a few teosinte plants from San Antonio and of teosintes from Durango and Chapingo are most readily explained by the assumption that these knobs have been derived from maize. The knobs on the chromosomes of all forms that seem to be pure teosintes are terminal except the one on chromosome VI adjacent to the point where it is attached to the nucleolus.

Even teosintes found in the wild are never in locations that exclude possible contamination with maize either recently or at some time in the past. Consequently any form that may appear as a connecting link between teosinte and maize is in danger of being explained by the possibility of maize having entered into its ancestry.

Throughout the study there was much to suggest that knobs are enlarged chromomeres, that they are constant in their position on a chromosome, and that B-type chromosomes have, along much of their length, abundant material similar to that making up the knobs of the normal chromosomes. The writer suggests that much of the abnormal associations of B-type chromosomes is due to the adherence of this knoblike material, a condition which also would explain the frequent apparent association of more than two chromosomes.

SUMMARY

A comparison of the morphological characters of the chromosomes of Florida teosinte with those of teosinte collected at El Progreso and Moyuta, in southern Guatemala, suggests a close relationship of the teosintes from these three sources. This indicated relationship confirms the historical record of the origin of the commercial teosinte now grown in Florida.

A direct comparison of the chromosomes of Florida teosinte with those of maize reveals slight differences between the homologues in length and also in the positions of the fiber-attachment regions. These differences are most noticeable in chromosomes V and IX. Florida teosinte and maize both have knobs on certain chromosomes, but in only two instances are these knobs located in corresponding positions in the two genera.

An indirect comparison of the chromosomes of teosinte collected at Nojoyá and San Antonio Huixta, in northern Guatemala, with those of the teosinte from southern Guatemala shows many characteristics in common. However, the short arms of two (and sometimes three) of the short chromosomes, which in the teosinte from southern Guatemala are without knobs, are found to terminate in knobs in the teosinte from northern Guatemala.

The occurrence of terminal knobs on the short arms of chromosomes VII and IX and of internal knobs on the long arms of chromosomes IV and VI suggests that the teosinte of northern Guatemala is more closely related to, or else more contaminated with, maize than is that of southern Guatemala.

Perennial teosinte (*Euchlaena perennis* Hitchc.) is a tetraploid species having very inconspicuous terminal knobs.

The chromosomes of Mexican teosinte from Durango and Chapingo have only a few knobs that would distinguish them from maize, which suggests that both types have resulted from recent hybridization of teosinte with maize.

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Disregarding the universally present internal knob on chromosome VI adjacent to its attachment to the nucleolus, the number and position of knobs on the nearer relatives of maize suggest the following classification:

Guatemalan teosintes:

(1) From southern Guatemala (Florida, Progreso, Moyuta). Prominent terminal knobs; diminutive terminal knobs infrequent.

(2) From northern Guatemala (Nojoyá and San Antonio). Prominent terminal knobs; frequent diminutive terminal knobs. Internal knobs and terminal knobs on the short arms of three of the short chromosomes, indicating contamination with maize.

Mexican teosintes:

(3) Perennial. Diminutive terminal knobs only.

(4) Durango. One terminal knob, linking it with teosinte. Many internal knobs, linking it with maize.

(5) Chapingo. Occasionally a small terminal knob, linking it with teosinte. Many internal knobs, linking it with a much-knobbed maize.

Knobs on the chromosomes of *Tripsacum* are terminal except the one on the chromosome that is attached to the nucleolus. This knob is universally present adjacent to the point of attachment in maize and all its near relatives.

Except for the knob on chromosome VI, near the nucleolus, all internal knobs on the chromosomes of teosinte may be taken as indications of contamination with maize.

The positions of knobs are found to be relatively constant on chromosomes in teosinte from Guatemala and from Mexico and in certain strains of maize from the Southwestern States.

It is suggested that knobs are enlarged chromomeres and that B-type chromosomes contain large quantities of knoblike material.

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RINGING AND FRUIT SETTING AS RELATED TO NITROGEN AND CARBOHYDRATE CONTENT OF GRIMES GOLDEN APPLES[1]

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INTRODUCTION

The failure of a large percentage of blossoming spurs to set fruit presents a problem of immense economic importance to apple growers and often is the cause of losses in orchards where pollination and varietal incompatibilities are not limiting factors. That a part of this failure may be due to conditions of nutrition within the plant has been recognized by many investigators as well as by practical fruit growers.

Much research work has been reported dealing with the internal composition of apple spurs, twigs, bark, and other portions of the tree as it affects blossom-bud formation. But little, if any, work has been reported dealing with the composition of the plant coincident with variations in the setting of fruit, and only a limited amount on the chemical composition during the period of fruit setting.

It is well established that early applications of readily available nitrogen fertilizers to bearing apple trees, low in nitrogen, greatly increase the percentage of blossoming spurs that set fruit. It has been reported that early ringing of branches on some trees may increase the setting of fruit, even on trees that may be benefited by applications of nitrogenous fertilizers. The first of these two treatments would be expected to increase the nitrogen content of the tree, and the second to increase the carbohydrate materials.

It has been assumed from common observation that young, vigorous apple trees fail to set as high a percentage of their blossoming spurs as do older bearing trees. This failure has been attributed to vigor of vegetative growth as opposed to fruitfulness. More recently it has been attributed to a lack of a proper balance between nitrogenous compounds within the tree in relation to carbohydrate accumulations.

The influence of vigor and conditions affecting nutrition upon the setting of fruit has been recognized by investigators who have studied pollination, fruit-bud formation, and other factors involved, yet the chemical phases of the problem during the period of fruit setting have been almost entirely neglected.

This study involved an attempt to vary the percentage of blossoming spurs on Grimes Golden apple trees that would set fruit, and to determine by chemical analyses some of the variations in composition that accompanied variations in the percentage of blossoms to set fruit. The influence of pollination as a factor was effectually elimi-

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[2] The author is indebted to Dr. W. H. Chandler, University of California, under whose general guidance this problem was carried; to Dr. H. R. Kraybill and J. T. Sullivan of the Purdue University Agricultural Experiment Station for suggestions relative to the chemical phases of the problem; and to D. R. Bowman, who made the microchemical determinations for soluble nitrogen and assisted in other analyses.

nated by hand pollination of the flowers with viable Jonathan pollen, or by using a larger number of trees open to the same conditions of open pollination, where it had been determined that hand pollination was not a more certain method of securing fertilization. The conditions of soil management, pruning, rootstocks, climate, and similar factors were the same in the two groups of trees.

MATERIALS AND METHODS

To study any existing correlation between the chemical composition of fruit spurs and their ability to set fruit, it is necessary to vary experimental conditions so that, as nearly as may be, one lot will set few fruits on blossoming spurs, and other lots may be expected to set fruits on a large percentage of blossoming spurs. To be most dependable, treatment should be given in the year of fruit setting.

Under natural conditions the percentage of blossoming spurs that will set fruit cannot be predicted. Obviously, the performance of spurs which are analyzed cannot be determined. A large number of treated trees are therefore necessary to provide abundant blossoming spurs and to overcome errors due to too small a population.

Of various methods of influencing fruit setting that had been previously employed, ringing vigorous young trees and applying nitrate fertilizers to less vigorous trees seemed to give most promise. The effects of pruning seemed too variable on trees of the age of those available. Defoliation prevents the functioning of the fruit spur. Weather conditions cannot be controlled. While soil management has been known to affect the percentage of set in some seasons, that effect is variable.

Of these methods, ringing branches on young vigorous 8-year-old trees was the only successful one tried.

THE TREES USED

The apple trees used in this study were all of the Grimes Golden variety growing in the orchards of the Purdue University Agricultural Experiment Station, at Lafayette, Ind.

In 1931 approximately 100 branches were ringed just prior to the opening of the blossoms on 8-year-old trees under clean cultivation and cover crop. Other branches were ringed or scored (using the same cuts as in ringing, but not removing the bark) at later dates, up to 2 weeks after blossoming. Branches similar in size, forks where possible, were selected as checks. All blossoming spurs were recorded. After the June drop the percentage of blossoming spurs that set fruit was recorded.

SAMPLING METHODS

Active growing parts of spurs produced during the current season were sampled as this younger tissue gave promise of yielding a clearer picture of the rapid changes that occur during fruit setting. No older portions were included. The spurs, including all of the current season's growth, were removed from the tree and taken to the laboratory for fractioning and preservation. The spur tissues were divided into four fractions (1) Spur leaves, those leaves produced on the cluster base only; (2) lateral vegetative shoot growth from the cluster base, including shoots and subtended leaves, designated as spur shoots in the tables; (3) blossoms and (or) fruits; and (4) cluster base, including the peduncle. All sampling was done between 9 and 11 a. m.

Samples were taken in 1931 on May 14 when petal fall was nearly complete, on May 19 as the first fruits were dropping, on May 25, and on June 2 when larger fruits were approximately five-eighths of an inch in diameter.

Each fraction was weighed separately and dropped into redistilled 95-percent alcohol that had been brought to a boil. The alcohol and sample were held at boiling temperature for 20 minutes. One-fourth gram, or more, of calcium carbonate was added to each sample, depending on the weight of the sample. The samples were cooled, tightly stoppered, and stored for later analysis.

In 1931, 19 representative trees were selected on which 2 or more branches had been ringed on either April 28 or 29. Each ringed branch had a comparable check on the same tree. Wherever possible a forking pair of branches was used. From 20 ringed branches and 20 check branches on these 19 trees, 5 spurs each were taken, 2 of these from the terminal on longer 1930 shoot growth, 2 from medium shoot or long spur growth, and 1 from a short spur. The resulting samples were quite uniform, especially at the first sampling.

Only such spurs as carried blossoms, or later fruits, were sampled. As has been stated, it is desirable to vary the percentage of spurs that set fruit as much as possible by treatment and study the variations in composition of the spurs sampled. If in later samplings, for the lower setting class, only spurs which had dropped their fruits, or which could be determined would drop their fruits, were taken, many subnormal and weak spurs would be included and results would not be representative. It is recognized that spurs carrying fruits, in the case of those setting a low percentage of their blossoming spurs, might be expected to more closely approach the composition of spurs that set their fruit than would completely nonsetting spurs. In addition, among the group of spurs that set fruit in a high percentage of cases will be included some spurs from which the fruits will later fall.

These conditions present a difficult problem to overcome and, even with the large variation in the percentage of blossoming spurs that set fruits between the two treatments in 1931, it is probable that the composition as found in each case is more nearly the average of the two classes than would be respective samples of spurs that were certain either to set fruit or to drop their fruits, as the case may be.

METHODS OF CHEMICAL ANALYSIS

EXTRACTION OF SOLUBLE MATERIAL

The preserved samples were extracted with 80-percent alcohol and the residues transferred to weighing bottles. The dry matter of the residue was determined on the whole mass after drying at 100° C. for 1 week. The dry matter of the extract was determined after evaporating a 50-cc aliquot and drying at 70° for 24 hours.

NITROGEN

Total nitrogen was determined for the alcohol-soluble and the alcohol-insoluble fractions. The soluble fraction was determined by the microchemical methods described by Sullivan and Horat (*13*).[3]

Alcohol-insoluble nitrogen was determined by the Kieldahl method.

[3] Reference is made by number (italic) to Literature Cited, p. 875.

SUGARS

The copper reduction method of Quisumbing and Thomas *(11)* was used for reducing substances and sucrose.

Sucrose determinations were made on duplicate 50-cc portions of the cleared solution by the enzyme-hydrolysis method. Calculations of sucrose equivalent to the reduced copper were made from the Quisumbing and Thomas tables for invert sugar, multiplying this value by the factor 0.95.

STARCH

Duplicate samples, usually of 1 g weight, were placed in a 200-cc Erlenmeyer flask, 100 cc of water added, and immersed in a boiling water bath for 30 minutes to gelatinize the starch. The solution was then cooled and 10 cc of a 1-percent taka diastase solution added, covered with toluol and placed in an oven, and held at a temperature of from 35° to 38° C. for 24 hours. At the end of this time the sample was immersed in a boiling water bath to inactivate the enzyme and remove the toluol. It was filtered while hot and the residue washed with hot water. The filtrate was cooled, cleared as for reducing sugar determinations, and made to volume. Concentrated hydrochloric acid was added to a 150-cc aliquot of this solution to make an acid concentration of 2 percent. The solution was then immersed in a boiling water bath for 2 hours, cooled, nearly neutralized with sodium hydroxide, and made to volume. The reducing power was determined on duplicate samples, as for sugars, and calculated as dextrose. The starch in each sample was determined by multiplying the dextrose value by 0.9.

RESULTS

An unusual percentage of blossoming spurs set fruit in Indiana in 1931 on most varieties and under most orchard conditions. 1930 was an extremely dry year, followed in the spring of 1931 with good weather during the blossoming period. These conditions have been observed to favor fruit setting. All treated and untreated trees probably set fruit more heavily than in an average year. Comparisons are therefore relative only. It could hardly be expected that so high a percentage of fruit setting could be secured on repeating the treatments in some other years.

EFFECT OF RINGING ON SETTING OF FRUIT

Ringing pronouncedly affected the setting of fruit in 1929 on young trees. Branches were ringed or scored between April 28 and May, 18 1931, in an attempt to produce a sufficient variation in fruit setting between the different treatments to provide material for chemical analysis. The results of the work are summarized in table 1. This table covers more than 10,000 blossoming spurs and indicates that ringing approximately doubled the percentage of spurs that set fruit and that scoring was also effective. In addition to increasing the number of spurs that set their fruits, ringing and scoring seemed to increase the average number of fruits per spur.

TABLE 1.—*Effect of ringing on the setting of fruits*[1] *on Grimes Golden apple trees, 8 years old, 1931*

Treatment and date	Ringed or scored				Check, no treatment			
	Spurs blossoming	Spurs set	Fruits set	Spurs set	Spurs blossoming	Spurs set	Fruits set	Spurs set
Ringed:	*Number*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Number*	*Percent*
Apr. 28, 29	1,075	953	1,626	88.6	780	396	471	50.7
Do	205	197	266	96.0	214	99	117	46.3
Do	849	716	1,287	84.3	551	235	298	42.6
May 1	1,029	784	1,111	76.1	990	341	401	34.4
Apr. 28	731	703	1,292	96.1	564	273	321	48.4
Do	489	458	793	93.6	484	205	253	42.4
Total	4,378	3,811	6,375		3,583	1,549	1,861	
Average			1.7	87.0			1.2	43.2
Scored:								
May 1	137	92	107	67.2	122	36	36	29.5
Do	329	205	255	62.3	271	123	139	45.3
May 4	364	236	273	64.8	401	157	165	39.1
Do	403	221	283	54.8	565	294	335	52.0
May 18	152	135	206	88.8	154	80	92	51.9
Total	1,385	889	1,124		1,513	690	767	
Average			1.3	[2] 64.2			1.1	[2] 45.6

[1] The setting of fruits was recorded after the June drop.
[2] Percentage based on totals.

In all of these data, where an excessively heavy blossoming occurred, the fruit drop was proportionally greater, regardless of treatment.

Table 1 indicates that fruit setting can be influenced over a considerable period of time during and following the blossoming period. Scoring on May 18 markedly increased the numbers of fruits that set. These data also indicate that ringing has relatively less effect on branches that tend to set fruit on a higher percentage of their blossoming spurs. This is more clearly indicated in table 2, showing fruits set on individual branches on trees from which chemical samples were taken.

TABLE 2.—*Fruit-setting data*[1] *for trees from which chemical samples were taken in 1931*

[Sample branches not included]

Tree no.	Branches ringed Apr. 29					Nonringed branches			
	Branch no.	Spurs blossoming	Spurs set	Fruits set	Spurs set	Spurs blossoming	Spurs set	Fruits set	Spurs set
		Number	*Number*	*Number*	*Percent*	*Number*	*Number*	*Number*	*Percent*
I–6	1	77	71	111	92.2	31	7	7	22.6
I–8	2	42	38	76	90.5	26	16	20	61.5
I–8	3	38	38	66	100.0	76	23	27	30.3
A–6	4	22	20	33	90.9	44	21	26	47.7
A–9	5	25	25	48	100.0	9	2	3	22.2
II–4	6	42	40	72	95.2	44	20	24	45.4
B–5	7	58	51	82	87.9	57	28	32	49.1
B–10	8	137	100	136	72.9	90	27	28	30.0
III–4	9	65	60	93	92.3	14	5	7	35.7
III–10	10	27	25	58	92.6	21	8	9	38.1
III–10	11	11	9	17	81.8	34	19	24	55.9
C–5	12	17	17	41	100.0	16	14	22	87.5
C–9	13	25	25	42	100.0	15	12	13	80.0
12–4	14	30	27	40	90.0	25	9	9	36.0
12–4	15	56	46	66	82.1	24	8	10	33.3
0–2	16	28	23	38	82.1	31	7	10	22.6
0–5	17	192	81	87	42.2	60	15	20	25.0
P–3	18	29	29	77	100.0	55	11	12	20.0
Total		921	725	1,183		672	252	303	
Average				1.6	[2] 78.7			1.2	[2] 37.5

[1] Fruit-setting data were recorded in July after the June drop was over.
[2] Percentage based on totals.

CHEMICAL ANALYSES

Fresh and Dry Weights

The total fresh and dry weights of the parts of the spur on the 1931 sampling dates are given in table 3. The leaf samples from ringed branches were slightly higher in both fresh and dry weights, although the dry weight, as percentage of fresh weight, was slightly less in these leaves on the first two sampling dates.

The vegetative lateral shoots from the cluster base were higher in dry weight on the first three sampling dates in those samples from ringed branches, although as a percentage of the fresh weight they were higher on the first, third, and fourth, and lower on the second. The total shoot growth on spurs from nonringed branches was somewhat greater on June 2.

The flowers and fruits on ringed branches were higher in fresh weight and in both total dry weight and dry weight as percentage of fresh weight.

The cluster base, like the flowers and fruits, was higher in fresh weight and contained less moisture in spurs on ringed branches.

TABLE 3.—*Fresh and dry weights of parts of 100 Grimes Golden apple spurs each from ringed and nonringed branches on 8-year-old trees, 1931*

Part of spur	Treatment of branch	Fresh weight				Dry weight			
		May 14	May 19	May 25	June 2	May 14	May 19	May 25	June 2
		Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Spur leaves	Nonringed	73.2	73.1	79.5	77.6	22.2	22.3	25.1	25.0
	Ringed	77.8	77.9	83.0	79.1	23.5	23.0	27.5	27.2
Spur shoots	Nonringed	21.3	36.5	52.0	74.1	6.2	11.2	16.0	24.2
	Ringed	20.7	39.5	54.9	68.3	6.5	11.4	17.4	23.3
Flowers and fruits	Nonringed	51.1	73.9	106.7	242.8	12.2	15.7	21.2	41.4
	Ringed	52.0	75.4	116.6	360.0	12.9	17.5	25.0	62.7
Cluster base	Nonringed	12.3	13.1	14.6	17.2	2.8	3.6	4.0	4.7
	Ringed	12.6	15.6	16.0	19.0	3.3	4.0	4.4	5.6
Total	Nonringed	157.9	196.6	252.8	411.7	43.4	52.8	66.3	95.3
	Ringed	163.1	208.4	270.5	526.4	46.2	55.9	74.3	118.8
		Moisture on fresh-weight basis				Dry weight on fresh-weight basis			
		May 14	May 19	May 25	June 2	May 14	May 19	May 25	June 2
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Spur leaves	Nonringed	69.7	69.5	68.5	67.7	30.3	30.5	31.5	32.3
	Ringed	69.8	70.5	66.9	65.6	30.2	29.5	33.1	34.4
Spur shoots	Nonringed	71.0	69.4	69.2	67.4	29.0	30.6	30.8	32.6
	Ringed	68.6	71.0	68.4	65.9	31.4	29.0	31.6	34.1
Flowers and fruits	Nonringed	76.2	78.8	80.1	83.0	23.8	21.2	19.9	17.0
	Ringed	75.2	76.8	78.6	82.6	24.8	23.2	21.4	17.4
Cluster base	Nonringed	77.3	72.5	72.5	72.8	22.7	27.5	27.5	27.2
	Ringed	73.8	74.3	72.4	70.7	26.2	25.7	27.6	29.3
Total	Nonringed	72.6	73.2	73.8	76.9	27.4	26.8	26.2	23.1
	Ringed	71.7	73.1	72.6	77.4	28.3	26.9	27.4	22.6

When all parts of the spur are included in the totals, ringing increased the fresh and dry weights, although the dry weight was only slightly increased on a percentage of the fresh-weight basis.

Ringing increased the weights of apple spurs, particularly the reproductive organs and the cluster base.

Carbohydrates

Table 4 lists the total amounts in grams of sugars and starch found in the parts of apple spurs in 1931.

Reducing substances were present in as great or in greater quantities in all parts of the spurs from ringed branches that set fruit on a large percentage of their blossoming spurs, than they were in spurs from nonringed branches that dropped a large percentage of their fruits.

Table 4.—*Total sugars and starch in parts of 100 Grimes Golden apple spurs from ringed and from nonringed branches, 1931*

Part of spur	Treatment of branch	Reducing substances				Sucrose			
		May 14	May 19	May 25	June 2	May 14	May 19	May 25	June 2
		Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Spur leaves	Nonringed	0.66	0.52	0.73	0.93	0.44	0.17	0.45	0.56
	Ringed	.80	.59	.93	1.15	.44	.18	.56	.56
Spur shoots	Nonringed	.06	.15	.30	.71	.09	.09	.21	.33
	Ringed	.08	.17	.36	.74	.09	.09	.19	.37
Flowers and fruits	Nonringed	.52	.41	.87	4.10	.14	.10	.24	.28
	Ringed	.64	.47	.96	5.90	.14	.14	.28	.47
Cluster base	Nonringed	.01	.01	.02	.07	.03	.02	.04	.06
	Ringed	.02	.02	.04	.10	.03	.03	.05	.05
Total	Nonringed	1.25	1.09	1.92	5.80	.70	.39	.95	1.23
	Ringed	1.54	1.24	2.30	7.90	.70	.44	1.08	1.45
		Total sugars				Starch			
		May 14	May 19	May 25	June 2	May 14	May 19	May 25	June 2
Spur leaves	Nonringed	1.10	0.69	1.18	1.48	1.40	0.98	1.31	1.45
	Ringed	1.25	.77	1.49	1.71	1.70	1.09	2.13	2.18
Spur shoots	Nonringed	.15	.24	.51	1.04	.22	.56	1.08	1.88
	Ringed	.17	.26	.55	1.11	.37	.67	1.41	2.11
Flowers and fruits	Nonringed	.66	.52	1.12	4.38	.54	.51	1.18	1.88
	Ringed	.78	.61	1.25	6.37	.62	.61	1.42	3.76
Cluster base	Nonringed	.04	.03	.06	.14	.05	.14	.24	.35
	Ringed	.04	.05	.09	.16	.19	.21	.36	.68
Total	Nonringed	1.95	1.48	2.87	7.03	2.21	2.19	3.81	5.55
	Ringed	2.24	1.68	3.38	9.35	2.88	2.57	5.31	8.74

Sucrose was present in smaller total amounts than reducing substances and varied little in the two sets of samples, except that the greater numbers of fruits present contained more sucrose on the ringed branches.

Total sugars follow the same trends as reducing sugars, because the latter dominate these totals.

Starch also follows the same trend as reducing substances, being highest in spurs from ringed branches. Starch appears in larger amounts than do the reducing substances in all spur leaves, spur shoots, and cluster bases, but generally in somewhat lesser amounts in all flowers and fruits.

Spur shoots contain a much smaller fraction of sugars than spur leaves and much less starch in the earlier period of growth. Large quantities of these substances are moved to the fruit during its development.

Potter and his coworkers (*10*) have called attention to the usual low starch content of bearing spurs and say that certainly this is not due to a lack of materials passing through it. They estimate that at the outset the apple gained at the rate of 3 mg of carbohydrates per day and that at a later date this accumulation rose to as high as 160 mg per day. "At certain seasons at least 100 times as much carbohydrate passed through the spur each day as the total amount it contained." These observations were made relative to analyses of spurs including 1-year-old wood of the spur with or without the cluster base of previous fruits borne.

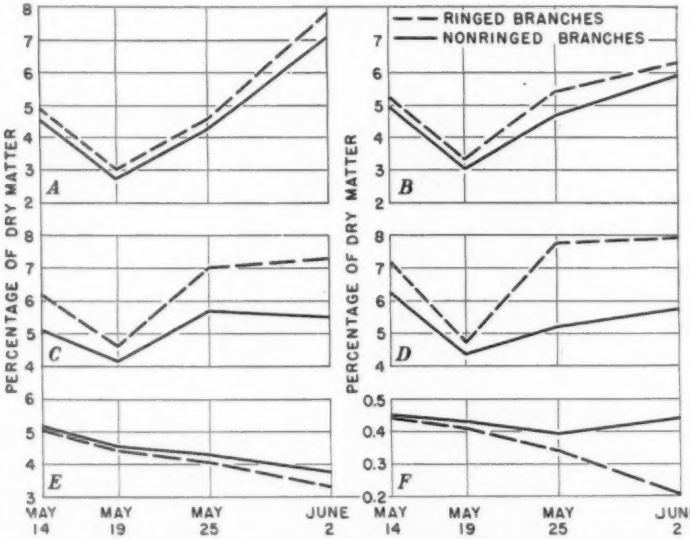

FIGURE 1.—Carbohydrate and nitrogen contents of Grimes Golden apple spurs and spur leaves between May 14 and June 2, as affected by ringing: *A*, Total sugars in spurs; *B*, total sugars in spur leaves; *C*, starch in spurs; *D*, starch in spur leaves; *E*, total nitrogen in spurs; *F*, alcohol-soluble nitrogen in spurs.

Table 5 and figure 1 show the sugars and starch as percentages of dry weight. On this basis the spur leaves, spur shoots, and cluster bases from ringed branches were as high as or higher in reducing substances than those from nonringed branches. The flowers and fruits from ringed branches were higher in reducing substances on May 14, about equal on May 19, and somewhat lower on May 25 and June 2, as compared with those of the nonringed group.

There was less sucrose in spurs from ringed branches on May 14, more on May 25, and less on June 2, as compared with nonringed. These differences are small and vary in different parts of the spur.

Total sugars in the spur as a whole were somewhat higher in the ringed than in the nonringed branches. Leaves from ringed branches were consistently higher in total sugars but this did not hold true for other spur parts.

TABLE 5.—*Sugars and starch as percentage of total dry weight in parts of 100 Grimes Golden apple spurs from ringed and from nonringed branches, 1931*

Part of spur	Treatment	Reducing substances				Sucrose			
		May 14	May 19	May 25	June 2	May 14	May 19	May 25	June 2
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Spur leaves	Nonringed	2.99	2.33	2.91	3.69	1.97	0.76	1.80	2.23
	Ringed	3.42	2.57	3.38	4.24	1.87	.76	2.03	2.05
Spur shoots	Nonringed	.98	1.33	1.85	2.93	1.49	.84	1.33	1.34
	Ringed	1.27	1.46	2.09	3.18	1.36	.79	1.07	1.57
Flowers and fruits	Nonringed	4.24	2.61	4.12	9.91	1.18	.66	1.14	.67
	Ringed	4.99	2.66	3.85	9.42	1.09	.81	1.13	.75
Cluster base	Nonringed	.47	.31	.52	1.56	.97	.53	1.06	1.37
	Ringed	.45	.45	.88	1.85	.81	.67	1.18	.97
Total	Nonringed	2.89	2.06	2.90	5.97	1.61	.73	1.43	1.26
	Ringed	3.34	2.22	3.10	6.65	1.51	.78	1.45	1.22
		Total sugars				Starch			
		May 14	May 19	May 25	June 2	May 14	May 19	May 25	June 2
Spur leaves	Nonringed	4.96	3.09	4.71	5.92	6.31	4.40	5.24	5.81
	Ringed	5.29	3.33	5.41	6.29	7.21	4.72	7.76	7.93
Spur shoots	Nonringed	2.47	2.17	3.18	4.28	3.53	5.06	6.73	7.77
	Ringed	2.63	2.25	3.16	4.75	5.64	5.82	8.10	9.08
Flowers and fruits	Nonringed	5.42	3.27	5.26	10.58	4.38	3.23	5.57	4.54
	Ringed	6.08	3.47	4.98	10.17	4.83	3.49	5.67	6.00
Cluster base	Nonringed	1.44	.84	1.58	2.93	1.74	3.87	5.95	7.43
	Ringed	1.26	1.12	2.06	2.82	5.71	5.26	8.06	12.24
Total	Nonringed	4.50	2.79	4.33	7.23	5.10	4.15	5.74	5.71
	Ringed	4.85	3.00	4.55	7.87	6.22	4.59	7.15	7.36

Spurs from ringed branches contained more starch in all their parts than did those from nonringed branches.

Tables 4 and 5 indicate that higher concentrations of carbohydrate material accompanied a more fruitful condition of the spur at the time of fruit setting. These tables also indicate a high transference of carbohydrates to the active spur, and especially to the fruits.

NITROGEN

Table 6 shows the amounts of alcohol-soluble nitrogen, alcohol-insoluble nitrogen, and total nitrogen stated both in grams and as percentages of dry weight in the spur parts. On May 14 and 19 the differences in absolute amounts of alcohol-soluble nitrogen per spur were not great. After that date the tendency seems to have been for the parts of spurs from nonringed branches to increase in soluble-nitrogen content more rapidly than those from ringed branches. The insoluble fraction and the total nitrogen, on the other hand, were, with one exception, practically as high or higher in all parts of the spurs on the ringed branches as on the nonringed ones.

TABLE 6.—*Nitrogen in parts of 100 Grimes Golden apple spurs from ringed and from nonringed branches, 1931*

Part of spur	Treatment	Alcohol-soluble nitrogen				Alcohol-soluble nitrogen on dry weight basis			
		May 14	May 19	May 25	June 2	May 14	May 19	May 25	June 2
		Grams	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*
Spur leaves	Nonringed	0.037	0.035	0.036	0.036	0.17	0.15	0.14	0.14
	Ringed	.042	.036	.035	.031	.18	.16	.13	.11
Spur shoots	Nonringed	.016	.024	.023	.029	.26	.21	.15	.19
	Ringed	.017	.021	.022	.023	.26	.19	.13	.10
Flowers and fruits	Nonringed	.107	.129	.160	.308	.88	.83	.75	.74
	Ringed	.111	.133	.160	.165	.86	.74	.64	.27
Cluster base	Nonringed	.034	.041	.040	.042	1.20	1.07	.99	.90
	Ringed	.034	.040	.036	.029	1.02	1.01	.81	.52
Total	Nonringed	.194	.229	.259	.415	.45	.43	.39	.44
	Ringed	.204	.230	.254	.248	.44	.41	.34	.21
		Alcohol-insoluble nitrogen				Alcohol-insoluble nitrogen on dry-weight basis			
		May 14	May 19	May 25	June 2	May 14	May 19	May 25	June 2
Spur leaves	Nonringed	1.133	1.007	1.080	0.997	5.11	4.51	4.32	3.98
	Ringed	1.189	1.038	1.134	.996	5.05	4.51	4.13	3.66
Spur shoots	Nonringed	.315	.498	.644	.829	5.09	4.47	4.02	3.43
	Ringed	.319	.504	.702	.727	4.94	4.41	4.05	3.12
Flowers and fruits	Nonringed	.473	.530	.727	1.134	3.89	3.39	3.43	2.74
	Ringed	.501	.603	.868	1.764	3.89	3.44	3.48	2.82
Cluster base	Nonringed	.103	.110	.118	.124	3.71	3.06	2.94	2.64
	Ringed	.110	.116	.121	.131	3.28	2.89	2.73	2.35
Total	Nonringed	2.024	2.145	2.569	3.083	4.67	4.07	3.88	3.24
	Ringed	2.120	2.261	2.825	3.618	4.59	4.04	3.81	3.05
		Total nitrogen				Total nitrogen on dry-weight basis			
		May 14	May 19	May 25	June 2	May 14	May 19	May 25	June 2
Spur leaves	Nonringed	1.170	1.041	1.116	1.033	5.28	4.66	4.46	4.13
	Ringed	1.232	1.074	1.169	1.027	5.23	4.66	4.26	3.77
Spur shoots	Nonringed	.331	.522	.667	.858	5.36	4.68	4.16	3.55
	Ringed	.336	.525	.724	.749	5.20	4.60	4.17	3.22
Flowers and fruits	Nonringed	.580	.659	.887	1.442	4.77	4.21	4.19	3.48
	Ringed	.613	.736	1.028	1.929	4.75	4.22	4.12	3.08
Cluster base	Nonringed	.137	.150	.158	.166	4.91	4.13	3.93	3.55
	Ringed	.144	.156	.157	.160	4.30	3.91	3.54	2.88
Total	Nonringed	2.218	2.372	2.828	3.498	5.12	4.50	4.27	3.67
	Ringed	2.324	2.491	3.078	3.866	5.03	4.45	4.15	3.26

Based on the percentage of dry weight, the nonringed branches carry spurs with a slightly higher nitrogen content, except that fruits remain fairly constant throughout the two treatments. The differences in percentage of nitrogen are not large and probably are accounted for in the differences in moisture content and total weight of the spurs. Percentages of total and of alcohol-soluble nitrogen in the spurs are shown in figure 1.

TRENDS SHOWN

Comparing the distribution of fresh and dry weights and the amounts of various substances in each part of the spur expressed as percentage of the total found in the spur, the tendencies shown are: (1) That the developing fruit draws more heavily upon the reducing substances and soluble nitrogen than do other portions of the spur;

and (2) proportionally more of the spur total of these substances accumulates in the fruits than would be assumed on a basis of the ratio the fruits bear to the total weight of the spur. With the possible exception of sucrose, which seems to accumulate more largely in the leaves, the various other substances appear to be distributed in about the proportions of the dry weight.

DISCUSSION

The results recorded show higher nitrogen content of parts of bearing spurs than do the analyses of Murneek (*8*, *9*), Schrader and Auchter (*12*), and Marsh (*6*), who have analyzed parts of spurs at a comparable season of growth activity. Schrader and Auchter (*12*) found a maximum of 3.24 percent of total nitrogen on a dry-weight basis in the blossoms and new growth of spurs on 26-year-old York Imperial trees in sod that had been treated with nitrogen fertilizers. Murneek reported 3.32 percent of total nitrogen in the flowers and fruit shortly after flowering in 1925, and 2.55 percent at about the same date in 1926. In the new growth he found 2.99 percent in 1925 and 3.74 percent in 1926. These samples were taken from 30-year-old Jonathan trees in 1926 and from the same trees in 1927. Murneek's (*9*) total fresh and total dry weights were higher than those recorded here. A closer agreement is found between the total amounts of nitrogen in 100 spurs of Murneek's tables and the 1931 data here presented. For example, on May 17, 1926, Murneek found 2.12 g of nitrogen in 100 Jonathan spurs at the beginning of the first drop. On the young Grimes Golden trees in Indiana on May 19, 1931, 100 spurs contained 2.37 g of nitrogen. Development seemed to have been more rapid, as judged by chemical composition, in Indiana in 1931 than in Missouri in 1926, although the trends are similar.

Howlett (*4*) collected samples of fruits about 1 week after full bloom in 1923 and found a maximum nitrogen content of 4.36 percent of dry weight in Roxbury Russet. The Grimes Golden fruits in Indiana in 1931 contained about 4.75 percent of nitrogen on the same basis at about the same period of development.

All of these results confirm Murneek's (*9*) conclusion that nitrogenous substances are moved into the bearing spur in abundance during fertilization and fruit setting.

Murneek (*9*) had the only comparable material including only new growth and sampled over a considerable period of time. He fractioned the spurs in a slightly different manner. "New growth" consisted of the cluster base with the usual side bud or a short shoot, never longer than 1 cm. Leaves included those directly attached to the cluster base and those on the bud or short shoot. Fruits are of course comparable. The spurs he examined averaged higher in fresh and dry weight. His total sugars were lower than in the Indiana Grimes Golden, but show similar trends.

Age of tree, variety, season, climate, methods of sampling, and analysis all markedly influence final results, and it is therefore of interest that the results of several workers do show similar trends and amounts of materials moving to the spurs.

In the work here reported a drop in percentage dry weight of reducing substances, sucrose, and starch occurred between May 14 and May 19 in the leaves of the cluster base and in the fruits. There was a drop in sucrose in all parts of the spur and a slight drop in starch in

the cluster base on ringed branches during this period. This reduction in carbohydrate fractions is in agreement with the work of Leclerc du Sablon (*5*), Murneek (*9*), Hooker (*3*), and Mitra (*7*). Whether it is significant as a factor in fruit setting is not apparent. It does indicate that during active growth large amounts of the carbohydrate storage materials are utilized in growth and respiration.

The set of fruit on young vigorous Grimes Golden trees was doubled by ringing the branches just before the bloom. This increased set was accompanied by an increase in sugars and starch and by a slight decrease in nitrogen content. This would indicate that in these young vigorous trees an additional supply of carbohydrates had accompanied an increased set of fruit. The differences in the percentage of nitrogen are small, although consistent, and probably represented only a reduction relative to the accumulation of carbohydrates. This is indicated by the fact that the total nitrogen by weight was higher per spur in the ringed branches, but with a higher dry weight they show a lower percentage than in nonringed branches.

The carbohydrate content of the spurs on these vigorous young trees was higher at the first sampling in 1931 than it was in the case of 13-year-old trees at a similar date in 1930; the nitrogen content was not materially different. It is also true that untreated trees, 8 years of age, set a higher percentage of their blossoming spurs in 1931 than did 13-year-old trees in 1930, but since different seasons and other variable factors were involved such correlations would hardly be significant.

Neither ringing nor the addition of nitrogen fertilizers increased the set of fruit on 13-year-old trees in 1930. These trees blossomed very heavily in that year and set fruit on about 30 percent of their bearing spurs. There was some frost damage to the earlier opened flowers.

A heavy crop of blossoms is often followed by a lighter setting of fruit. It is possible that these trees set a maximum that could be expected under the conditions of a heavy bloom combined with somewhat adverse climatic factors. It would not be surprising, then, that neither ringing nor nitrogen applications would increase the percentage of blossoming spurs to set fruit.

Heinicke (*2*) reported that both ringing and application of nitrogenous fertilizers increased the set of fruit on both vegetatively vigorous and vegetatively weak trees. He recorded the bloom as "good." It is apparent that, for reasons not clear, ringing and the application of nitrogenous fertilizers before full blossom may not increase the set in all cases, at least beyond the June drop.

In another report Heinicke (*1*) described the effects produced by placing excised twigs in solutions of sugar and in solutions of sodium nitrate. In the sugar solution flowers often set and began to form fruits under greenhouse conditions, while in the sodium nitrate solution the flowers opened, in spite of injury to the leaves, were smaller, paler in color, and produced dwarfed stamens with poorly germinating pollen.

Carbohydrate accumulations, or the power within the plant to supply an abundance of these substances through photosynthetic activity, seem to be of importance in preventing the excessive dropping of fruits.

SUMMARY

Nutritional factors accompanying the setting of fruits on blossoming spurs of young vigorous Grimes Golden apple trees included a high level of carbohydrate synthesis and storage, and a relatively high level of available nitrogen.

Early ringing of branches on young vigorous trees doubled, or more than doubled, the percentage of blossoming spurs that set fruit in 1929 and 1931. These rings healed over rapidly during the active growing season.

Spurs on these ringed branches were higher in sugar and starch content than were spurs on nonringed branches.

When a large percentage of spurs on a branch blossomed, a relatively small percentage of such spurs set fruit.

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